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CEREAL CHEMISTRY

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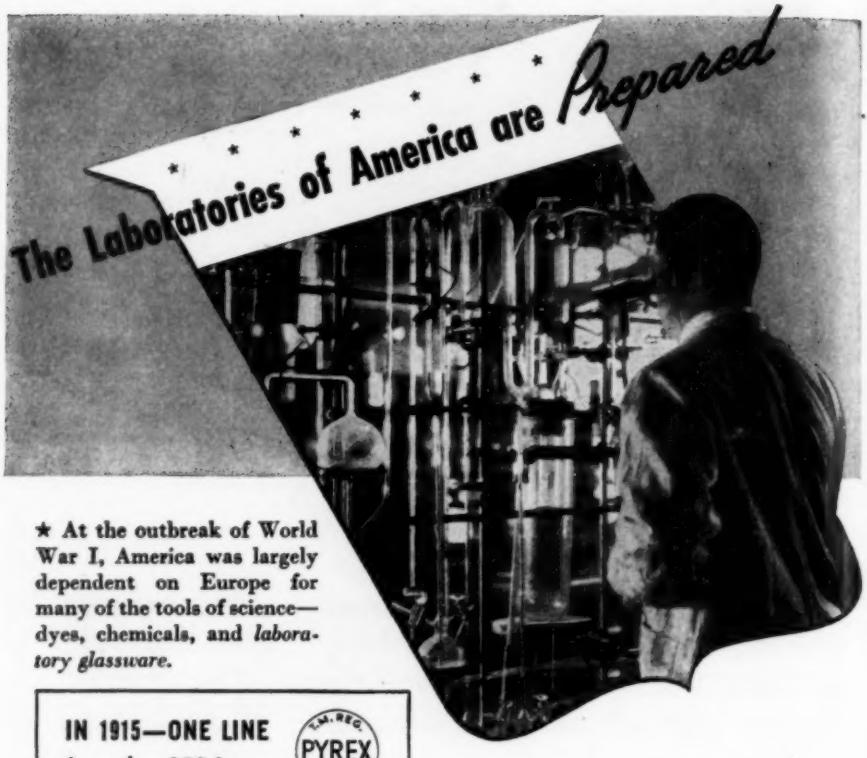

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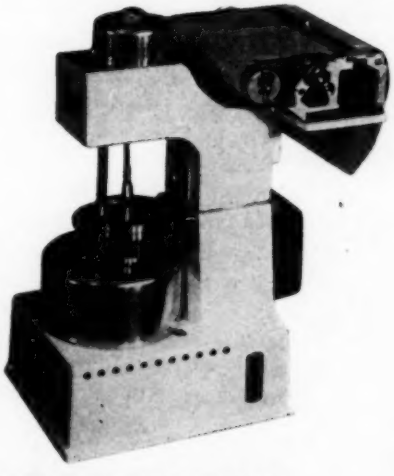

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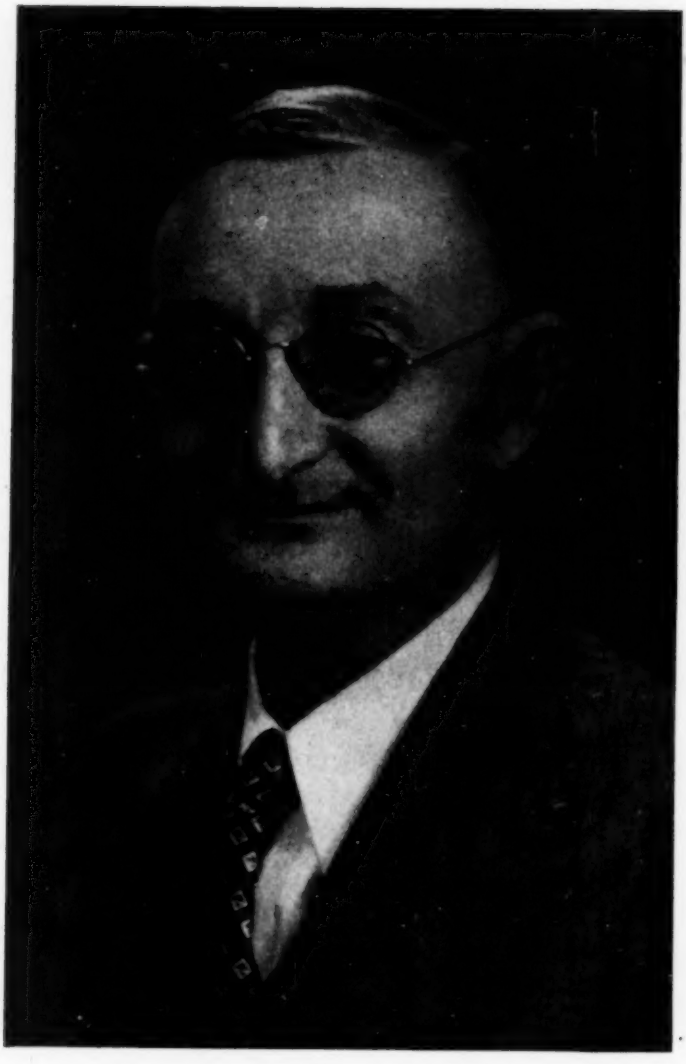
1885-1942



ROSS AIKEN GORTNER

CEREAL CHEMISTRY

VOL. XX JANUARY, 1943 NO. 1

ROSS AIKEN GORTNER

1885–1942

Cereal chemistry lost one of its most productive and distinguished votaries with the passing of Ross Aiken Gortner on September 30, 1942. Educated soundly in the fundamental sciences as an undergraduate, and subjected to rigorous training as a graduate student in physical and organic chemistry, Dr. Gortner became one of the pioneers who really created the science of biochemistry. His associations with the staff of the Station for Experimental Evolution during the five years following the completion of his graduate study served to introduce him to the biological sciences. Such colleagues as J. Arthur Harris, Blakeslee, and Davenport effectively expanded the scope of Gortner's scientific experiences to include the broad biological field. Moreover, this training was rigorous and intolerant of anything savoring of scientific inexactness.

This was the background of education and experience that Dr. Gortner brought to bear upon his biochemical activities at the University of Minnesota subsequent to 1916. In this environment it was inevitable that he should be confronted with problems in the cereal chemistry field which challenged his interest. The result was a series of studies supervised or engaged in by him which attempted to apply fundamental chemical and physical principles to the interests and needs of the cereal chemist and the cereal industries. A series of papers appeared in various scientific journals detailing the results of these scientific studies. In fact the first issue of *Cereal Chemistry* contained a paper from his laboratory, and the March issue of the same year carried his description of the determination of flour viscosity, which precipitated as much activity in this field as any contribution of the period. A list of Dr. Gortner's publications pertaining to cereal chemistry was published in a supplement to the November, 1942, issue of this journal, which describes the presentation of the Osborne Medal to him. This bears testimony to the breadth of his interests and the significance of his contributions.

As Chief of the Division of Agricultural Biochemistry for a quarter century, Dr. Gortner made the acquaintance of scores of scientists.

Many of these he came to know through his participation in the work of numerous scientific organizations. Others came to the University of Minnesota to meet this productive scientist in his own laboratory and to discuss problems of mutual interest. Still others began their advanced training in his Division, and pursued their early researches under his direction. He participated in the two short courses for cereal chemists offered at the University of Minnesota in 1923 and 1924, in the extension course conducted at the same institution at the request of the Northwest Section of the American Association of Cereal Chemists in 1929–30, and in the Minnesota Institute of Cereal Chemistry in February, 1938. In fact, no event of that nature held in the State would have been quite complete without him.

Many honors and distinctions came to Dr. Gortner in recent years, among them the conferring of the Thomas Burr Osborne Medal by this Association in May of this year. At that time his contributions to cereal chemistry and related fields were reviewed, and he was afforded an opportunity to present his philosophy of education and research. It was a timely presentation in these days of accelerated tempos of teaching and industrial investigations. For he emphasized and established from his experience and that of his students the soundness of programs based upon fundamentals, and necessitating adequate training in the basic sciences.

Dr. Gortner will be missed from many council halls and scientific groups. His interests were wide, and his contributions were correspondingly varied. Despite the distractions of these extensive tasks, he never lost the kindly and genuine personal interest which characterized his relations to his colleagues and students, however. The recollection of this friendship, of the advice which he gave, and of the clear elucidation of his views and findings will remain as a happy memory in the minds of many men and women who profited from knowing him.

C. H. Bailey

A COLLABORATIVE STUDY OF RIBOFLAVIN ASSAY METHODS[1]

JOHN S. ANDREWS

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1942)

The introduction of enriched flour and bread as commercial products was accompanied by numerous problems, not the least of which was that of methodology. Laboratories concerned with production control and regulation were confronted with the necessity of deciding which assay methods could be advantageously and appropriately applied. This was not easy in view of the rather uncertain position occupied by many of the available methods for evaluation of the vitamin content of cereals. In recognition of this situation the Research Corporation, acting through Dr. R. R. Williams, has sponsored several committees for the purpose of collaboratively examining assay procedures. This report deals with the activities of the Riboflavin Assay Committee.

This committee was organized on the occasion of the 1941 meeting of the American Chemical Society at St. Louis. It was comprised of nearly fifty individuals who had expressed interest in the problem and a desire to participate in its activities. Dr. F. E. Randall was appointed chairman, and he in turn selected six of the members to act as an advisory group on the selection of the collaborative methods. Each member of the advisory committee was furnished with two samples and requested to assay them by methods employed in his laboratory. One sample was a patent flour and the other the same flour containing added riboflavin. The collaborative method finally selected was to be chosen on the basis of performance with respect to these two flours.

Before this preliminary study was completed, Dr. Randall, because of conditions entirely beyond his control, found it necessary to resign the chairmanship of the committee. At Dr. Williams' request the writer agreed to take over this responsibility. The preliminary program was continued by assembling and examining the values reported by the advisory committee for the two flour samples. The assay methods employed were of two general types, fluorometric and microbiological, several modifications of each being represented. Table I summarizes the microbiological data.

Each of the methods presented employed the Snell and Strong (1939) procedure in that it used the *Lactobacillus casei* organism.

[1] Paper No. 45, Journal Series, General Mills, Inc., Research Laboratories.

TABLE I

MICROBIOLOGICAL ASSAYS OF COLLABORATIVE SAMPLES EMPLOYED IN THE PRELIMINARY STUDY OF METHODS

Collaborator	Method	Riboflavin content		Recovery of added riboflavin
		Patent flour	Fortified flour	
		μg/g	μg/g	μg/g
A	Scott, Randall, Hessel (1941)	0.50[1]	2.82	2.32
	Snell and Strong (1939)	0.40	0.90[1]	0.50[1]
B	Andrews, Boyd, Terry (1942)	0.40	3.00	2.60
	Modified Snell and Strong[2]	0.30	1.60[1]	1.30[1]
C	Andrews, Boyd, Terry (1942)	—	2.90	—
D	Cheldelin and Williams[3]	0.32	2.80	2.48
E	Andrews, Boyd, Terry (1942)	0.35	3.27	2.92
	Average	0.35	2.96	2.61

[1] Omitted from averages.
[2] Personal communication, Dr. R. A. Sullivan.
[3] Personal communication, Dr. V. H. Cheldelin and Dr. R. R. Williams.

Modifications introduced either different extraction techniques or different culture media. Examination of the data demonstrates that with one exception there is fairly good agreement on the sample of patent flour. If the value of 0.50 submitted by collaborator A is omitted the average riboflavin content is 0.35 ± 0.05 μg per gram. Thus most of the procedures might be considered acceptable. The values for the fortified flour are somewhat more erratic. Two are obviously out of line, suggesting inadequacies of the methods employed. If these are omitted the flavin content ranges between 2.80 and 3.27 μg per gram and averages 2.96. The difference between the averages for the two flours is 2.61 or 13% less than the 3 μg per gram which was actually added to the patent flour.

On the basis of these results the entire collaborative group was asked to make their assays on autoclaved, aqueous extracts which had been digested with takadiastase and filtered. Except for this modification the method of Snell and Strong (1939) was recommended. In addition the collaborators were also asked to submit values obtained by any other microbiological procedure with which they had had experience. In this way it was hoped that data for all the available methods would be accumulated and thus afford a comparison with a single, mutually employed procedure.

Three advisory collaborators submitted values obtained by fluorometric methods. Their results are summarized in Table II.

The average values shown in Table II are in very good agreement with those obtained microbiologically. The very small differences can readily be attributed to experimental error since even duplicate

TABLE II

FLUOROMETRIC ASSAYS OF COLLABORATIVE SAMPLES EMPLOYED IN THE PRELIMINARY STUDY OF METHODS

Collaborator	Method	Riboflavin content		Recovery of added riboflavin
		Patent flour	Fortified flour	
		μg/g	μg/g	μg/g
A	Hodson-Norris (1939)	0.50	3.04	2.54
B	Conner-Straub (1941)	0.35	2.70	2.35
C	Combination of both	0.26	3.00	2.74
	Average	0.37	2.91	2.54

assays by a single procedure will not usually show such concordance of results. This observation encouraged a belief that both microbiological and fluorometric methods were capable of satisfactory application to cereal products.

Selection of the preferred fluorometric method by the committee was not easy to make on the basis of the submitted data, since the differences between the results obtained by the three procedures were not regarded as significant. While the results reported by collaborator B for the patent flour agreed best with the average of all three collaborators as well as the average microbiological value, the assays obtained for the fortified flour by A and C were closer to that calculated from the amount of added riboflavin. If any of the methods possesses serious inherent weaknesses they are not detectable as far as the analyses of patent and riboflavin-fortified flours are concerned. Decision to recommend a combination procedure such as was employed by collaborator C was based on independent observations made in the assay of bread. As will be seen later this material yields extracts which present added problems in the fluorometric assay. As much as possible of the nonflavin pigments must be removed to avoid erroneously high values. The combination method appeared to be most effective for accomplishing this objective. This involved acid extraction of the sample, digestion with takadiastase, decolorization with permanganate and hydrogen peroxide, and adsorption on "florisil." The eluted riboflavin was determined by measuring the fluorescence before and after the addition of a known amount of riboflavin. The "blank" was determined by reducing the flavin with hydrosulfite.

The collaborators were asked to study this procedure and compare their results with those from the same procedure in which the permanganate treatment was omitted. This was done for the purpose of establishing the behavior of the oxidation both as regards pigment

removal and possible destruction of riboflavin. In addition they were requested to submit values obtained by any other fluorometric method they might be regularly using.

This represented a rather ambitious program, the complete fulfillment of which required considerable work on the part of the collaborators. The pressure of other activities together with interrupted schedules resulting from the war effort has prevented many from participating as they had originally planned. Twenty-three of the forty-eight collaborators found it impossible to make any contribution, while many of the remaining group were able to submit only those results which they obtained from their regularly employed procedures. Only a very few of the collaborators completed the collaborative program. For this reason this report is not as comprehensive a study of riboflavin methods as it was first hoped. It must be viewed not as the "last word" on the present status of methodology but rather as a preliminary effort. Many of the points brought out in the present study need further examination and cannot be unqualifiedly accepted without more complete confirmation.

The collaborative assays were carried out on three samples: whole wheat flour, whole wheat bread, and dried yeast. Considering that the work was being carried out primarily for examining methods applicable to the enriched flour program some wonderment might be expressed at this selection of samples. It would at first glance seem more logical to choose the two samples employed by the advisory committee, together with white bread containing some form of added riboflavin. The fortified flour, however, presented complications. This was brought out in the preliminary study. Failure to obtain the theoretical result could be just as easily attributed to lack of uniform distribution of the added riboflavin as to errors in methods. In the case of the white bread this objection would be absent but another factor might militate against entirely trustworthy results. Riboflavin in white bread has been shown to be quite sensitive to decomposition by light (Andrews, Boyd, and Terry, 1942), and while this problem deserves consideration in any assay study it presented a complication which it seemed best to avoid in the initial program. Then too there appeared to be some advantage in comparing bread with the flour from which it was made. Knowledge of the riboflavin content of the bread ingredients could be applied to an evaluation of the bread assays. For these reasons whole wheat products were chosen. It was felt that if methods were satisfactory for these products they would be equally satisfactory for the other materials. The sample of dried yeast was included largely as a control. Other collaborative studies, particularly that carried out by the A.O.A.C. (Kemmerer, 1941), have

indicated that a variety of methods are adaptable to this type of sample.

In considering the collaborative results each of the three samples is taken up separately. The fluorometric and microbiological methods are divided into two groups: those recommended by the committee and those employed independently by the collaborators. No attempt has been made to keep separate the results reported by the individual laboratories, all data being consolidated in these divisions. In a few instances some of the assays have been omitted entirely either because they were obviously in error or because the collaborator failed to identify sufficiently the method he employed. In other instances assay values have been omitted in the tabulation but have been considered in the discussion. This was justified on the basis that their inclusion would be misleading about the applicability of the other procedures.

The data reported for the sample of whole wheat flour are presented graphically in Figure 1. The histograms show the distribution of values, the open columns representing the microbiological, and the solid columns the fluorometric. No microbiological data are graphically included under "other methods" since only two assays fell in this group.

Considering first the "committee's methods," a rather wide variation is found in both the fluorometric and microbiological assays. Fluorometric values ranged from about 40% below to about 40% above the average, while those obtained microbiologically exhibited similar tendencies. There is, however, a better grouping of the microbiological data, and if the two values at the extreme right are omitted as being erroneously high, the agreement between the various analysts can be considered as fairly satisfactory. Due to these two values, however, the standard deviation is rather large and amounts to nearly 20% of the average value. Despite this it is less than the standard deviation of the fluorometric values, which is 0.328. In view of the relatively small number of assays this difference cannot be regarded as significant, and mathematically speaking neither method can be said to be superior to the other. Taking these variations into account it is interesting to note the close agreement between the averages. As determined microbiologically the flour contains 1.14 μg per gram, while fluorometrically the value is 1.12 μg per gram.

Fluorometric values shown under the heading of "other methods" were reported by seven collaborators. The methods represented included those published by Hodson and Norris (1939) and Conner and Straub (1941), together with two revealed in correspondence with Dr. Frey of the Fleischmann Laboratories and Dr. Kishlar of the Ralston-

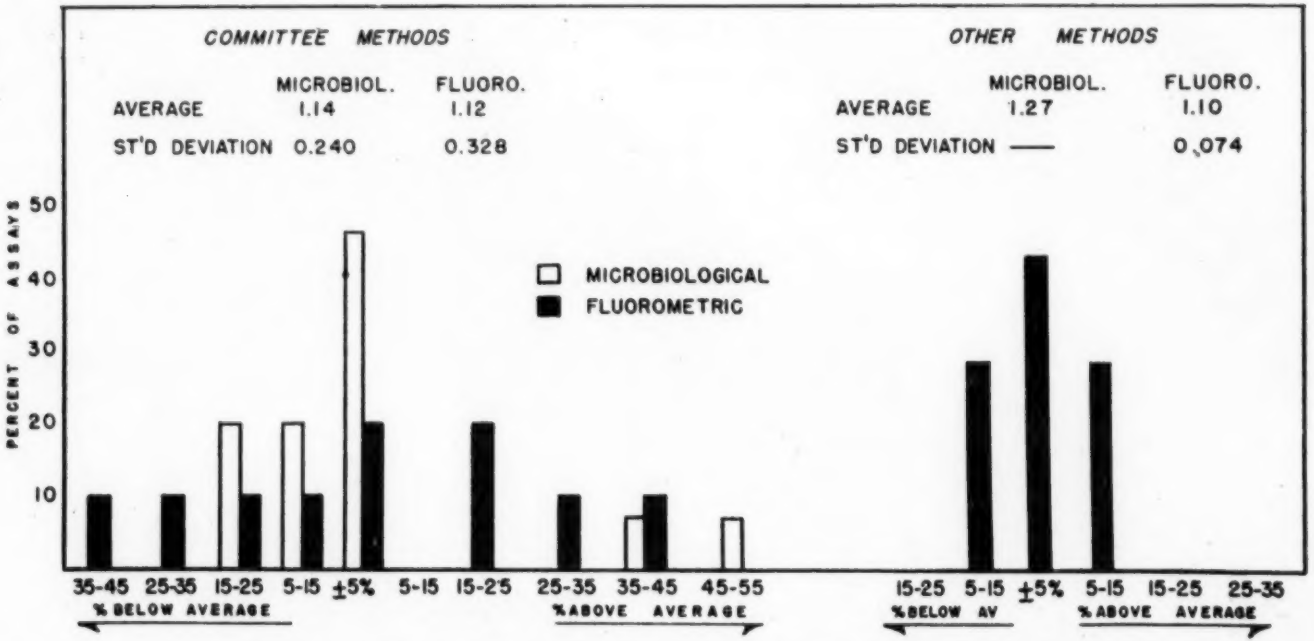


Fig. 1. Collaborative study of riboflavin assay methods—whole wheat flour, sample No. 1.

Purina Company. The average of these values, 1.10 μg per gram, is almost identical with that obtained by the "committee" method; but the agreement between laboratories is much better. It is believed that this observation cannot be used in judging the merits of the methods but rather expresses the greater skill and experience of the analysts concerned. The standard deviation for these "other" fluorometric methods is 0.074 and is significantly different from the value 0.24 found by the committee procedure.

The two microbiological values obtained by the "other methods" were 1.32 and 1.22 μg per gram. The average of 1.27 is somewhat higher than that obtained by the committee method. One of these values was obtained by using an acid extraction instead of the aqueous procedure suggested by the committee. The other compensated for nonflavin growth factors by incorporating photolyzed alkaline extract of bread in the culture medium. To what extent these methods represent improvements over that described by the committee cannot be decided on the basis of available data.

The results obtained from the sample of whole wheat bread are presented in Figure 2. Once again only two microbiological values were reported by the "other methods" and for this reason are not included in the histograms.

Turning first to the assays obtained by the "committee's methods" a rather wide variation in the results is again seen. The range of the fluorometric values is from about 30% below to 50% above the average. The microbiological data vary only slightly less. Standard deviations are 0.543 and 0.361, respectively, but the difference is not significant for the small number of assays considered. There does, however, seem to be a tendency toward greater uniformity in the microbiological procedure since 70% of the values reported can be considered to fall within a reasonable experimental error. This is not true of the fluorometric results.

The averages of the two types of methods are in quite good agreement. The fluorometric value of 1.70 μg per gram is only 6% higher than the 1.60 μg per gram found microbiologically. While, except for the flour, no actual analyses were made of the bread ingredients, some indication of the probable correctness of the bread assays can be calculated. Of the other ingredients used, only the yeast contributed riboflavin. Assuming that this yeast contained about 20 μg per gram, the 2% employed should increase the dried bread approximately 0.4 μg per gram over the riboflavin content of the flour. This also assumes that no gain or loss of riboflavin occurred during the baking processes in accordance with the findings reported by Andrews, Boyd, and Terry (1942) and the Technical Committee of the Millers' National

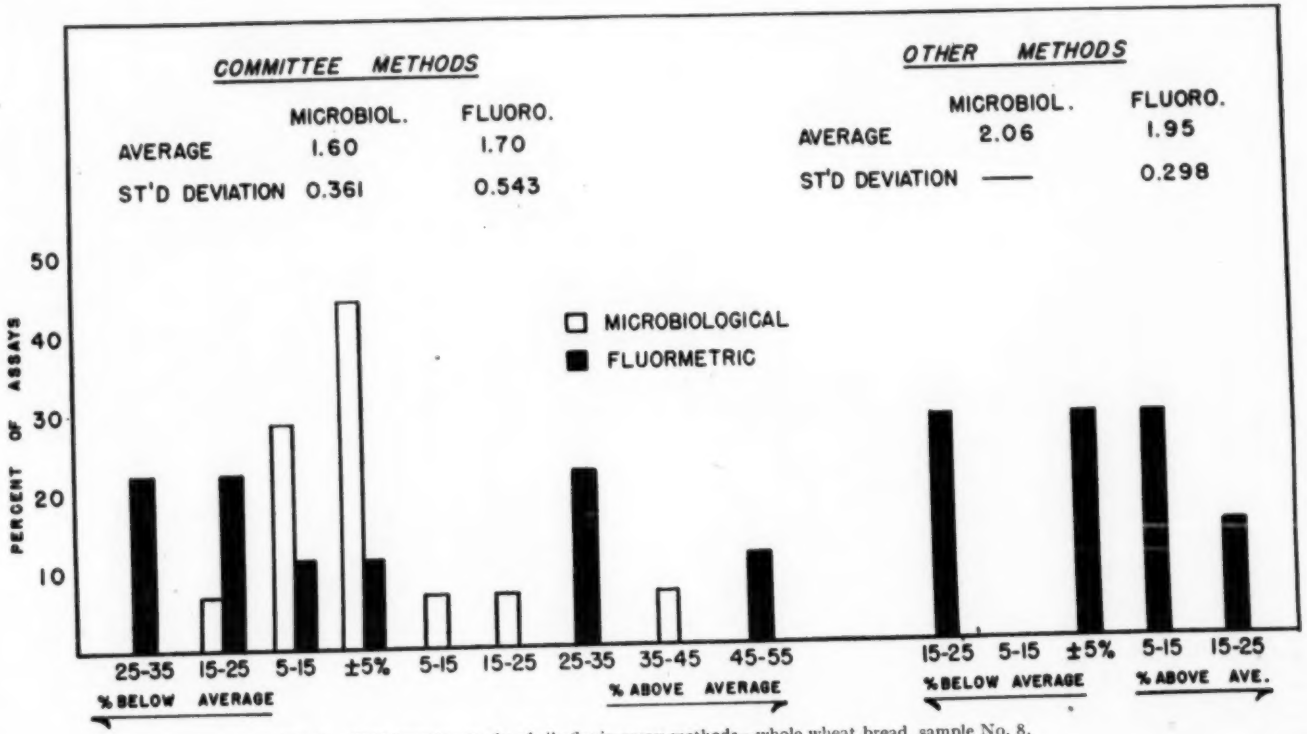


Fig. 2. Collaborative study of riboflavin assay methods—whole wheat bread sample No. 8.

Federation.[2] The actual increase found microbiologically amounts to 0.46 μg per gram and, fluorometrically, 0.58. On this basis the microbiological values would appear to be more nearly correct.

The fluorometric values obtained by the "other methods" for the sample of bread do not show the agreement exhibited in the case of the flour. A considerably greater spread as indicated by the standard deviation of 0.298 is observed. It is believed that this is due to the effect of bread pigments, which tend to introduce greater errors in the actual fluorometric readings. Unfortunately the collaborators' reports in many instances failed either to record their fluorescence data or to carry out the two analyses which were requested. This has prevented an evaluation of the influence of pigments on the assay. The limited data available indicate that excessively high blanks must be eliminated before reliable results can be assured. This point needs further study.

While statistical calculations show no significant difference between the "committee" and "other" fluorometric methods, it is obvious that the latter average is higher than that of the former. Two of the collaborators employed procedures which gave values nearly identical with the average found by the committee method. One of these was the Hodson and Norris (1939) procedure and the other that described by Dr. Kishlar.[3] The average was substantially increased, however, by inclusion of values obtained by the method of Conner and Straub (1941). These assays appeared to be high, due to the inclusion of fluorescence impurities in the final value calculated as riboflavin. A further indication of the failure of this method to record accurate values for bread can be seen in the differences between the bread and the flour. Three collaborators reported an average difference of 1.14 μg per gram, which is much above that supplied by the yeast. One other method was also reported but the results were omitted from consideration. This method gave a much lower value for the bread than it did for the flour.

The two "other" microbiological methods applied to the flour sample were also employed in the assay of the bread. Both gave essentially the same values, 2.02 and 2.10 μg per gram. The average, 2.06, is somewhat higher than that obtained by the "other" fluorometric methods. If the averages obtained for bread and flour by these two procedures are compared, the bread is found to be nearly 0.8 μg per gram higher. This is considerably more than the 0.4 which was calculated.

The assays reported for the sample of dried yeast are represented in Figure 3. The values obtained by the "committee's methods" for

[2] "Enriched Flour," special series No. 14. Published by Wheat Flour Institute, 309 West Jackson Blvd., Chicago.
[3] Personal communication.

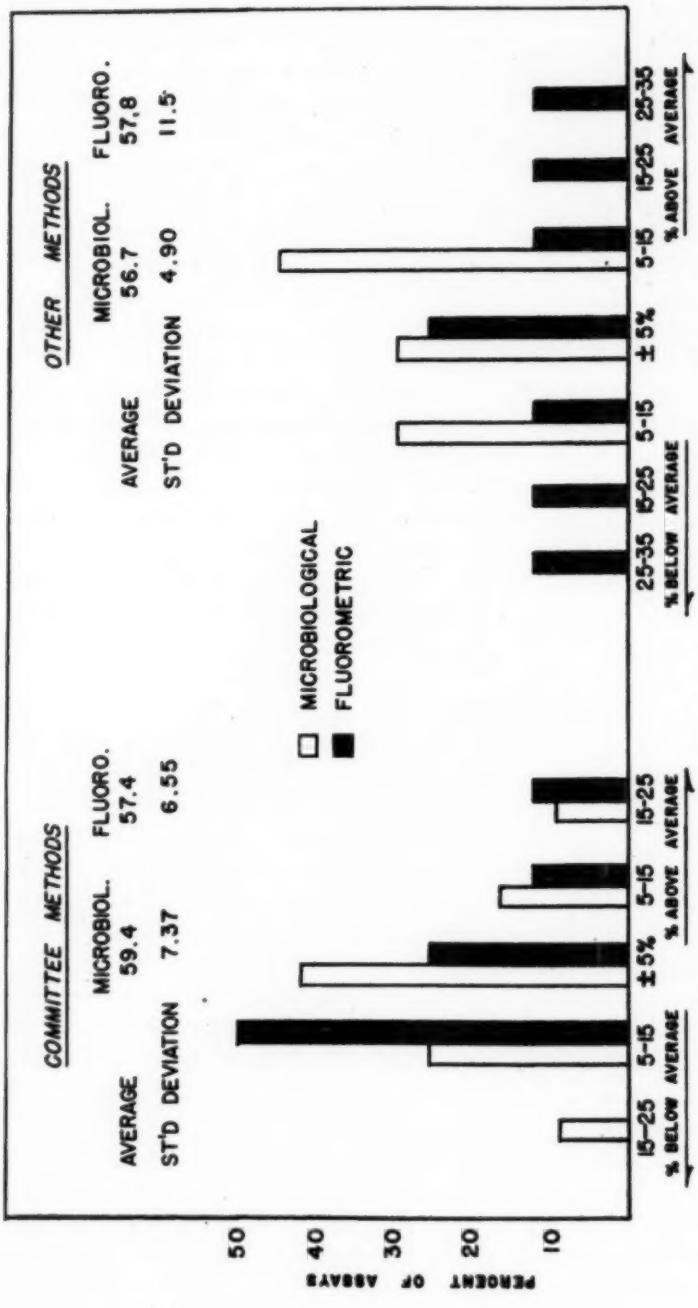


Fig. 3. Collaborative study of riboflavin assay methods—dried yeast sample No. 6.

this sample are seen to be much superior to those obtained for the cereals. This suggests that fewer difficulties attend its analysis. Both the fluorometric and microbiological procedures appear to be equally satisfactory since the averages are almost identical. The standard deviations, too, are very similar and represent no significant difference.

Strangely enough the fluorometric values obtained by the "other" methods show less uniformity for the yeast than for the cereals. The assays vary plus and minus 35% from the average, an equal number falling on either side. The fact that the average of 57.8 is almost exactly the same as that found by the "committee's method" suggests that no major source of error is inherent. Discrepancies between laboratories must be attributed to variations in technique. One laboratory reported a very low value not only for the yeast but also for the cereals. The analyst attributed his results to destruction of riboflavin by the treatment with permanganate. This is not borne out, however, by the other collaborators since their values obtained with permanganate decolorization were in good agreement with those obtained microbiologically.

Microbiological values obtained by the "other" methods show better uniformity than the corresponding fluorometric assays. While there is but little difference between the averages, 56.7 against 57.8 μg per gram, there is less scattering. The standard deviation of 4.90 is significantly different from that of 11.5 for the fluorometric methods. On the other hand there is no significant difference between the "committee" and "other" methods, either microbiological or fluorometric.

On the basis of these collaborative findings no clear-cut decisions can be made about the question of satisfactory riboflavin methodology. It appears that several methods are capable of yielding analogous results, and since some of these differ quite radically in principle it seems probable that the values are approximately correct. However, this conformity is not absolute proof of accuracy since several collaborators reported essentially identical values by fluorometric and microbiological methods but did not agree with each other on the actual magnitude. For this reason more experience is necessary before one or more of the methods can be given the final stamp of approval. Meanwhile the individual analyst must decide, on the basis of the available information and his own experience, just which method is most satisfactorily adapted to his own problem. Unfortunately riboflavin assays of cereals are not as readily confirmed as those for thiamin, where reliable animal assays are available. While some information about biological values exists in the literature it is far from satisfactory in its unanimity and comprehensiveness. One of the present collaborators attempted to apply a chick assay method to the preliminary

collaborative samples. He reported that the relatively low riboflavin content prevented any reliable evaluation either of the patent or riboflavin-fortified flour.

A few additional points brought out in the collaborative study should be included in this report. In most instances they are observations noted by one or more of the collaborators and are pertinent to a specific procedure. Thus, for example, two laboratories suggested that the use of permutit in the combined thiamin-riboflavin method of Conner and Straub (1941) resulted in a lowering of the riboflavin assays. Their data did not indicate whether this was due to adsorption of the flavin on the zeolite or to improved clarification of the final extracts.

Another observation dealt with the use of hydrosulfite. Here again several collaborators reported similar findings. Each noted that the addition of the recommended quantity of hydrosulfite was insufficient to reduce completely the fluorescence of the blanks in the nonpermanganate fluorometric procedure. Several times this quantity was required before successive additions produced a constant fluorescent reading. On the other hand, one collaborator indicated that when the permanganate oxidation was used the recommended quantity was entirely adequate. Since this amount of hydrosulfite is sufficient to reduce completely the riboflavin it is obvious that nonflavin, reducible impurities were present in relatively large amounts. Under such conditions it may be of advantage to use the Hodson and Norris (1939) technique of carrying out a preliminary hydrosulfite reduction before any fluorescence measurements are taken.

Commenting on this subject, two collaborators reported that sodium hydrosulfite produced a yellow color in the presence of pyridine-acetic acid. This could not be prevented by redistillation of the pyridine. The belief was expressed that this color adsorbed some of the blue incident light, thereby adversely affecting the fluorometric readings of the blanks. This would account for the lower blank values obtained when successive increments of hydrosulfite are added.

One collaborator expressed the belief that methods employing pyridine should be avoided if possible, since the objectionable odor is a practical handicap to many laboratories. For this reason and also because the adsorption on florisil prolongs the time required for analysis, he prefers some other procedure.

Two collaborators employed methods which depend solely on permanganate for decolorization and hydrosulfite for determining the blank. They reported assay values essentially in agreement with those yielded by the committee method, which combined the permanganate and florisil treatments. This suggests that the use of

florisil may perhaps be eliminated for many types of cereal samples. Further study will be required, however, before such a procedure can be specifically recommended.

One collaborator reported that the size of sample had a marked effect on the assay results. Smaller sample weights successively increased the riboflavin values until a maximum was reached. His data indicate that one-gram samples are more completely extracted than the five-gram quantities recommended by the committee. In the case of products which are relatively low in riboflavin, the use of florisil offers a convenient means for concentrating the eluates. Larger aliquots of the extracts can be passed through the florisil column, increasing the amount of adsorbed flavin. Elution with the usual amount of medium yields eluates having appreciably increased fluorescence. By this method as little as one gram of patent flour can be satisfactorily assayed. This collaborator found that such a sample gave a value of 0.45 μg per gram in contrast to 0.3 μg per gram obtained when 10 g of patent flour was used. The sample of whole wheat flour gave values of 0.8, 1.1, and 1.3 μg per gram for 10-, 5-, and 1-gram samples, respectively. This observation may have an important bearing on the selection of riboflavin assay methods.

That the effect of sample size is not confined to the fluorometric assay and is therefore probably an actual extraction problem was indicated by similar observations reported for the microbiological procedure. The same laboratory increased the riboflavin assays 10% to 20% by using 2½-gram instead of 10-gram samples. Lower sample sizes were not studied since microbiological assays cannot be obtained without concentrating the extracts.

Several collaborators reported values obtained by the original method of Snell and Strong. Their use of suspensions or untreated extracts yielded widely varying results. Values for the flour ranged between 1.1 and 4.3 μg per gram and for the bread, 2.0 to 8.0 μg per gram. The yeast assays on the other hand compared favorably with those obtained by other methods. The average of four assays was 56 μg per gram. This is further confirmation that cereal products require special consideration.

Two laboratories employed the method described by Scott, Randall, and Hessel (1941). This procedure, which requires long digestion with relatively large amounts of takadiastase and assay of the resulting homogenized suspension, gave erratic results. For one collaborator it yielded a difference of 0.16 μg per gram between the bread and the flour. For another the same difference amounted to 2.05 μg per gram.

Another collaborator recommended that slightly improved accuracy can be achieved in the fluorometric method if the galvanometer is adjusted to zero with the solvent blank before readings are taken.

Several laboratories raised the question of the relative merits of aqueous vs. acid extraction in the microbiological assay. Some opinion was expressed, but no evidence was presented, that the use of acid resulted in more complete removal of riboflavin from the cereals. This problem needs solution, but the answer is not easily determined. Complications arise from the changed composition of the assay medium and make it difficult to decide whether the increased growth of the organism is the result of higher concentrations of riboflavin or of the presence of nonflavin growth factors produced by the acid. This question requires further study.[4]

Two collaborators reported that it might be desirable to add nicotinic acid or the amide to the culture tubes employed in the microbiological assay. Their data, however, are somewhat conflicting, since in one instance the reported improvement in the agreement between the different assay levels was in sharp contrast to the wide variance noted in the other. Nor were the average assay values reported for the flour and bread essentially different from those reported by the other collaborators. The merit of adding nicotinic acid or one of its biologically active derivatives cannot be adjudged from available evidence.

The Collaborative Committee's Methods

A complete description of the recommended procedures, together with a detailed discussion of the reasons for their selection, is best afforded in the two communications addressed to the collaborators. For this reason they are reproduced below.

To the members of the Research Corporation Collaborative Committee on Riboflavin Assay Methods for Cereals

On October 14 the writer sent to each member of the Committee a communication describing the program for the collaborative study of riboflavin methods for cereals and promising that definite procedures would be recommended within a few weeks. The present letter describes these procedures and discusses some of the factors which led to their selection.

As indicated earlier it is not anticipated that these methods will represent the last word in methodology since ultimate perfection awaits the results of further research. Rather they should be viewed as attempts to utilize present information, particularly with respect to the adequacy of known methods for the riboflavin assay of cereals. Emphasis is placed on the cereal nature of the samples being examined since such products present problems which are not encountered in the assay of more concentrated materials.

Selection of the methods herein described has taken into consideration the several methods already reported in the literature. The fluorometric procedure combines some of the principles employed by Hodson and Norris (J. Biol. Chem. **131**:

[4] A recent paper presented by F. M. Strong and L. E. Carpenter before the 1942 meeting of the American Chemical Society at Memphis recommends acid extraction followed by adjustment of the pH to 4.5, filtering and ether extraction. The latter step removes fat-soluble factors which influence growth of the test organism as noted by Bauernfeind, Sotier, and Boruff (1941).

621, 1939) and Conner and Straub (Ind. Eng. Chem., Anal. Ed. **13**: 385, 1941), while the microbiological procedure is that of Snell and Strong (Ind. Eng. Chem., Anal. Ed. **11**: 346, 1939) to which has been added a detailed description of the preparation of the extracts.

It is hoped that each collaborator who can possibly do so will analyze the samples by both of these procedures. In addition it is requested that any other method familiar to the collaborator also be applied. Reports of the assay results should include a critical evaluation of the analyst's observations together with any suggestions for improvements. Wherever possible such suggestions should be accompanied by evidence of their applicability.

Decision to include the microbiological method in this collaborative study has been based on the relatively large number of laboratories which are employing this type of procedure. Preliminary reports indicate that more than half of the 48 collaborators will carry out microbiological assays. For this reason it is important to examine this method collaboratively.

While such products as yeast, dried milk, etc. can apparently be satisfactorily assayed by the Snell-Strong procedure, cereal products present a somewhat different picture. Nonflavin factors are present in sufficient concentration to influence the growth of the lactobacillus organism and adversely affect the assay results. Two general means of overcoming these factors have been suggested. One is a modification of the culture medium to compensate for these factors and the other is a treatment of the extracts to remove or inhibit their influence.

Dr. Frey has kindly revealed a compensatory procedure which is being successfully used in the Fleischmann Laboratories. This employs an alkaline photolyzed bread suspension which is added to the medium for both the unknown and reference standard. Another method which is being studied in several laboratories increases the concentration of sugar in the medium above that originally proposed. A third method being examined in the writer's laboratory involves the removal of flavin from a portion of the cereal extract by adsorption on fullers earth or "florisil" and the addition of this extract to the riboflavin standards.

Two procedures have been used for removing or inhibiting nonflavin factors. One of these, described by Bauernfeind, Sotier, and Boruff at the 1941 convention of the American Chemical Society, requires a preliminary solvent extraction for separating growth-stimulating fatty substances. The other reported by Andrews, Boyd and Terry at the Atlantic City Convention and by Scott, Randall and Hessel (J. Biol. Chem. **141**: 325, 1941) digests the sample with takadiastase during extraction.

The method being presented to the collaborators describes the use of the enzyme treatment. This selection has been made primarily because it is simple to use, gives results comparable with those obtained fluorometrically and has been used quite successfully in several different laboratories. Any collaborator who is employing some other modification is urged to compare it with this procedure.

Extraction is carried out in an aqueous medium since water appears to be as effective as dilute acids for removing riboflavin from cereals when enzymatic degradation of the starch is employed. This avoids the necessity of subsequently neutralizing the extracts and minimizes the possible effect of salts on the growth of the test organism. A rather extensive comparison of aqueous and acid extraction as applied to wheat flour and bread has shown that the use of water gives more uniform assay values. Acid extraction occasionally leads to erratic results which are higher than those obtained either from aqueous extracts assayed microbiologically or acid extracts measured fluorometrically. The fact that aqueous and acid extractions give practically identical results by the fluorometric procedure suggests that the acid may promote the formation of nonflavin factors which adversely affect the microbiological assay.

Autoclaving is recommended since experiments demonstrate that no measurable loss of riboflavin occurs in the presence of cereals. Less drastic heating, however, may be equally efficient since a few experiments indicate that temperatures as low as 60°C in the presence of takadiastase give the same assay values. It is not felt, however, that this observation has been sufficiently established to warrant recommendations at the present time.

The time of treatment with takadiastase is kept to a minimum to avoid possible changes in riboflavin content which might result from the action of contaminating bacteria and excessive formation of sugar. The effectiveness of takadiastase appears to be related to its liquefying action on starch rather than its saccharifying action. The digestion period recommended is believed to be adequate. The quantity of takadiastase is rather small but is as efficient as larger amounts. All takadiastase

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preparations apparently contain some riboflavin and where large quantities are used correction for this riboflavin content may become necessary. Hence, uncontrolled excesses should be avoided.

It is important to note that clarification calls for *filtration* and not centrifugation. Filtered extracts consistently give more uniform assay values and are free from globules of fat which, particularly in the case of bread, are frequently present in centrifuged extracts. The use of filtration appears to offer a convenient means of avoiding fatty compounds which may interfere in the assay.

The proposed method is as follows:

A 5-gram sample of the whole wheat flour or bread is *uniformly* suspended in approximately 90 ml of distilled water using a 300 ml Erlenmeyer flask (see Note 1). This suspension is then autoclaved for 15 minutes at 15 lbs pressure. After cooling to about 50°C 1 ml of freshly prepared 6% takadiastase solution (see Note 2) is stirred in and the mixture allowed to stand for three-quarters of an hour. It is then transferred to a graduated cylinder and made up to 100 ml with distilled water. Clarification is accomplished by filtering (see Note 3), the first 10 to 15 ml of filtrate being discarded. Aliquots of the filtrate are assayed by the method described by Snell and Strong (Ind. Eng. Chem., Anal. Ed. **11**: 346–50, 1939) (see Note 4).

All operations are carried out in a semidarkened room (drawn window shades) and the suspensions and solutions are kept covered with a black cloth except when being handled.

Notes:

1. Samples of flour tend to lump when mixed with water. Care should be taken to insure the absence of large lumps prior to autoclaving.
2. The product manufactured by Parke, Davis and Co. has been found satisfactory in enzymatic activity and freedom from significant quantities of riboflavin in the amounts recommended.
3. Whatman No. 1 or No. 40 filter paper retains no measurable amount of riboflavin.
4. Growth of the organism should be measured by titration rather than determination of turbidity. The latter is influenced by cereal proteins which may precipitate during the fermentation.

In reporting results the collaborators are requested to include all analytical data (*i.e.* quantities of extracts used, titration values, etc.) and a copy of the standard riboflavin curve. Since the present collaborative study is directed particularly to the methods for cereals, no specific recommendations are being presented for the sample of dried yeast. A.O.A.C. collaborative studies have demonstrated that microbiological and fluorometric methods give comparable results with this type of product. With respect to the former procedure, extracts can be prepared by autoclaving suspensions in water and using them directly or by autoclaving with 0.1*N* HCl followed by neutralization. The writer believes that clarity of the extracts may have some effect on the uniformity of assay results and for this reason suggests that filtration be employed. Also, acid extraction may possess an advantage since filtration yields clearer extracts than when water alone is used.

The fluorometric method which is being recommended has been devised to minimize the adverse influence of cereal pigments, especially those present in bread as the result of caramelization and dextrinization during baking. The quantitative removal of these pigments by chemical means is difficult and uncertain and it has appeared undesirable to present a procedure which relies solely on such processes. Similarly, physical methods for separating riboflavin from other pigments cannot be regarded as entirely effective. A combination of physical ("florisil" or "supersorb") and chemical (permanganate) methods such as are employed by Conner and Straub (1941) is the most effective which has come to the writer's attention. For many products this procedure appears to be entirely satisfactory.

For the assay of bread, however, there are indications that the removal of nonflavin pigments may be incomplete, thereby introducing appreciable errors into the analytical results. For this reason some modification is required at least for the bread sample which is being examined.

The method introduced by Hodson and Norris (1939) depends less on the separation of nonflavin pigments than on techniques designed to compensate for their influence on the fluorometric observations. By measuring the fluorescence of extracts before and after the addition of a known amount of pure riboflavin, the fluorescence of the unknown flavin is evaluated under the actual conditions of assay.

Then by adding hydrosulfite to completely reduce the riboflavin the "blank" of the cereal extract is obtained. Preliminary treatment of the extract with hydrosulfite followed by oxidation with air is carried out to strengthen the assumption that nonflavin reducible substances do not produce erroneous "blank" values.

While this procedure has proved quite satisfactory for a variety of materials, some samples, particularly bread, have been observed where obvious erratic errors are introduced. It has occurred to us that these errors might be modified by combining the purification method of Conner and Straub with the fluorescence-measurement procedure of Hodson and Norris. Unfortunately, however, the permanganate decolorization with hydrogen peroxide is not easily combined with the treatment with hydrosulfite and for this reason purification has been confined to adsorption on "florisil." (This adsorbing agent is another name for the "supersorb" first described by Ferrebee in J. Clinical Investigations **19**: 251–6, 1940. It can be obtained from the Floridin Company, Warren, Pa.)

The use of such a method for the assay of bread has resulted in significantly lower values for the "blanks" obtained by the Hodson-Norris procedure and greater uniformity in replicate analyses. Breads containing known amounts of riboflavin have yielded values which are in quite good agreement with the theoretical. For these reasons the collaborators are requested to follow the details presented below and to compare their results with those obtained by other fluorometric procedures which they are regularly using.

Simplification of procedure has been attempted wherever it appeared possible. No standard riboflavin curve is required since the assay results are calculated directly from the fluorescence readings and solid hydrosulfite is used to avoid the inconvenience of preparing fresh, cold solutions. Careful control of the quantity of hydrosulfite is unnecessary as long as the flavin is completely reduced (this is readily ascertained by observing the fluorescence change which occurs) and greatly excessive quantities are avoided. The latter is important since high salt concentrations may change the fluorescence properties of the "blanks."

In order to serve as a check on the concentration of the collaborators' solutions of pure riboflavin it was earlier planned to submit a solution of sodium fluoroscein to each member with the request that he or she report the fluorescence ratio between these two solutions. While fluorescein and riboflavin do not have identical fluorescence properties it was thought that the ratio between these two might afford a satisfactory comparison for evaluating the riboflavin standards. However, it is now known that filters, even when carrying the same identification marks and supplied by the same instrument maker, are not always sufficiently similar to justify such a procedure. Differences which give rise to 15% variations in the above ratios have already been observed.

Accordingly, plans for this phase of the collaborative study have been dropped for the present. Each collaborator should use his own set of filters with the expectation that small differences will not significantly influence the results.

As in the microbiological method precautions should be taken to prevent undue exposure to light. Assays should be carried out in a semidarkened room and extracts and eluates kept covered with a black cloth when not in actual use. Filter paper should be checked to demonstrate its freedom from significant adsorptive properties. This can be conveniently checked by comparing the fluorescence of the standard riboflavin solution before and after filtration. The quantity of takadiastase used in the extraction has been reduced, for reasons presented above, to a minimum compatible with ease of filtration. Measurements of fluorescence should be carried out as rapidly as possible to avoid destruction of riboflavin.

Details of the collaborative method are as follows: A 5-gram sample of the bread or whole wheat flour is autoclaved at 15 lbs pressure for 15 minutes with 50 ml of 0.1*N* sulfuric acid. After cooling to about 50°C, the suspension is quickly neutralized to a pH of approximately 6 with a 6.5% $Na_2PO_4 \cdot 12\ H_2O$ solution (this requires approximately 12 ml). One ml of freshly prepared 6% takadiastase solution is then added and allowed to stand for three-quarters of an hour in the dark. After making up to a total volume of 100 ml, the mixture is filtered, the first 10 to 15 ml of filtrate being discarded.

A 20-ml aliquot of the filtrate is passed through a column (see Note 1) of florisil. After the 20-ml portion has completely passed through the column, the effluent is discarded and the column washed with 5 to 10 ml of hot distilled water. It is then dried by drawing air through the capillary end of the tube. The absorbed riboflavin

is then eluted with 15 ml of 20% pyridine in 2% acetic acid (see Note 2), the eluate being collected in a 25-ml glass-stoppered graduate. It is then made up to a total volume of 20 ml with the pyridine-acetic acid solution and thoroughly mixed. If cloudy, this must be filtered before making fluorometric measurements.

A 14-ml aliquot of this eluate is pipetted into the cuvette of the fluorophotometer (see Note 3), and the fluorescence (A) determined. Prior to this measurement, the light intensity of the fluorophotometer is standardized by means of a solution of sodium fluorescein (see Note 4). The concentration of this fluorescein solution will depend upon the purity of the sodium salt. A considerable variation has been found in commercial products. Concentration should be such that its fluorescence will be approximately the same as that of a solution containing 0.2 μg/ml of pure riboflavin and will range somewhere between 0.025 and 0.1 μg/ml. Adjustment of the light intensity is made by means of the iris to give a galvanometer deflection of 25 divisions (see Note 5). Light filters are 351 for the fluorescent light and a 511–038 combination for the incident light.

Following the determination of the fluorescence of the eluate (A), 1 ml of solution containing 1.5 μg (see Note 6) of pure riboflavin in 20% pyridine-2% acetic acid is added to the contents of the cuvette with stirring and the resultant fluorescence (B) determined. A few crystals of solid sodium hydrosulfite (10 to 20 mg) are added and the solution stirred until the riboflavin color has been destroyed (or the hydrosulfite dissolves). The fluorescence (C) is then recorded.

The riboflavin content of the sample is calculated by means of the following equation:

$$\frac{A - 1.07C}{B - .93A} \times 0.1 \times \frac{100 \text{ ml}}{5 \text{ g.}},$$

where the values 1.07 and .93 are dilution factors (see Note 6); A, B, and C are the galvanometer deflections in scale divisions; and 0.1, the concentration of the added riboflavin per ml.

Notes:

1. The tubes containing this florisil have an inside diameter of 6 mm, fitted at the lower end with a capillary which, when the column contains approximately 1 gram of florisil, gives a rate of flow through the column of about 1 ml per minute. The top of the tube is belled to contain 15 to 20 ml of solution. The height of the actual florisil layer is approximately 2½ inches.

2. Mallinckrodt's "Analytical Reagent" quality pyridine has been found to be satisfactory without further purification. However, each collaborator should check his pyridine for fluorescence and if it is appreciable should purify by distillation.

3. This quantity is recommended where the Pfaltz and Bauer instrument is employed. Other instruments may require other quantities depending upon the capacity of the cuvette.

4. Sodium fluorescein solution, because of its greater light stability is preferred to riboflavin solutions for standardizing the light intensity.

5. Once again, this is based on the Pfaltz and Bauer instrument.

6. Where cuvette capacities require other than 14 ml of the eluate this concentration should be adjusted to add an amount of riboflavin equivalent to 0.1 μg per ml of total solution. Similar volume corrections will be required in the "dilution factors" used for calculating the final results (15/14 = 1.07, 14/15 = 0.93).

Details of the methods described should be carefully followed to insure that the collaborators' assays will be comparable from the standpoint of methodology. Results of replicate analyses should be reported individually and include all fluorometric readings. Data derived from other methods are also requested in order to permit a comparison of the various procedures.

Several collaborators have raised questions about the condition of the samples which they earlier received, expressing fears that storage may have changed the content of riboflavin. If the samples have been stored under the conditions recommended in Dr. Frey's letter of August 22 there should be no concern on this score. Unless unduly exposed to light or excessive moisture to produce mold the samples should be entirely satisfactory. Reserve supplies are available, however, and can be sent to any member who believes his present samples are unsatisfactory.

Sincerely yours,
JOHN S. ANDREWS

To the Members of the Research Corporation Collaborative Committee on the Riboflavin Assay Methods for Cereals

On December 1 the writer sent you a description of the two procedures recommended for the collaborative study of riboflavin assays. In discussing the fluorometric method bread was indicated as offering difficulties due to the presence of excessive amounts of foreign pigments. The use of florisil as a means of partially separating these pigments was described. It was pointed out that the treatment with permanganate followed by hydrogen peroxide, while not entirely effective, gave the best removal of interfering substances but that its use was not easily combined with the subsequent measurements of fluorescence.

At the time the procedure was being studied experiments were carried out by applying the permanganate oxidation *prior* to the adsorption on florisil. The results did not appear to be very promising since low and erratic assay values were obtained. We now recognize that this difficulty arose from our failure to properly consider hydrogen ion concentrations.

Further study has indicated that when this is done the technique shows sufficient promise to justify your consideration in the collaborative program. "Blank" values, while still higher than those of the reagents, are appreciably lower than those obtained by the recommended procedure, and for this reason errors can be reduced to a minimum in "low potency" breads where foreign-pigment concentration is high in proportion to riboflavin. While the bread sample which was sent to you is not of the "low potency" type (relatively speaking) and for this reason not so subject to errors, I believe you will want to try the procedure which appears to be more generally applicable. Accordingly I am describing it below.

A 5- to 10-g sample of bread is suspended in 50 ml of 0.1*N* sulfuric acid and autoclaved for 15 minutes at 15 lbs of pressure. While still hot 12 ml of 6.5% $Na_3PO_4 \cdot 12\ H_2O$ is added and when the temperature reaches about 50°C. 1 ml of 6% takadiastase is stirred in. After standing about three quarters of an hour the suspension is made up to 100 ml and filtered, the first 10–15 ml of filtrate being discarded. To a 25-ml aliquot of the filtrate is added 0.5 ml of glacial acetic acid and 1 ml of 4% potassium permanganate solution. After one minute 2 ml of 3% hydrogen peroxide is added to decolorize the excess permanganate. The total solution (28.5 ml) is passed through the column of florisil and after washing and drying the riboflavin is eluted with about 20 ml of 20% pyridine in 2% acetic acid. The eluate is made up to 25 ml with the pyridine-acetic acid and a 14-ml aliquot is taken for the fluorescence examination previously described.

Since this procedure uses the same filtered extract as that described on page 10 of my letter of December 1 and differs only in the introduction of the oxidation treatment prior to adsorption on the florisil, both methods can be applied to the same solution. In order to demonstrate the influence of different amounts of foreign pigments on the final assay I am requesting each collaborator to carry out both procedures. I believe that such a comparison will be quite instructive and add considerably to the value of the collaborative study.

Sincerely yours,
JOHN S. ANDREWS

Summary

Microbiological and fluorometric methods for the assay of riboflavin have been collaboratively studied. As applied to whole wheat flour, whole wheat bread and dried yeast, each of these two types of methods gave comparable assays. Various modifications of the microbiological procedure yielded average values of 1.15, 1.65 and 58.4 μg per gram for the air-dried samples of flour, bread and yeast, respectively. Several types of fluorometric procedures gave the following corresponding averages: 1.11, 1.81 and 57.6 μg per gram. The major discrepancy appeared in the case of the bread sample where incomplete removal of pigments is believed to interfere in certain of the fluorometric assays.

Despite the general agreement between the microbiological and fluorometric methods there is a wide range between the values reported by the individual analysts. All the methods studied showed this tendency, thereby adding to the difficulty of selecting the one best adapted to the assay of cereals. In general the microbiological procedure gave somewhat better uniformity of results but fluorometric methods showed sufficient promise to justify equal consideration. A greater variety of procedures is offered by the fluorometric method, thereby extending the scope of investigations essential to its evaluation.

The collaborative study has brought out numerous factors which influence the assay results. Particularly in the fluorometric procedure several experimental details, prerequisite to satisfactory performance, have been revealed. These factors are discussed in the light of the collaborators' comments.

Acknowledgments

The author wishes to express his appreciation to Messrs. Harold M. Boyd, David E. Terry, and Donald Holm, who assisted not only in experimentally studying various procedures to aid in the selection of the methods finally proposed by the Committee, but also checked on the validity of many of the observations made by the collaborators. He wishes also to acknowledge the assistance of Dr. Frank C. Hildebrand with the statistical analyses and of the following individuals who served on the advisory committee preliminary to the actual collaborative study and also participated in the subsequent collaboration: V. H. Cheldelin, Summit, New Jersey; R. T. Conner, General Foods Corp., Hoboken, N. J.; O. L. Kline, Food & Drug Administration, Washington, D. C.; L. C. Norris, Cornell University, Ithaca, N. Y.; F. E. Randall, Coop. G. L. F. Mills, Inc., Buffalo, N. Y.; H. C. Schaefer, Ralston Purina Co., St. Louis, Mo.; E. E. Snell, University of Texas, Austin, Texas; R. A. Sullivan, Kraft Cheese Co., Chicago, Ill.

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Additional reports were obtained from two of the collaborators too late to be included in the tabulations presented above. Their data are reported here: Using the committee's microbiological method one collaborator obtained the following values: whole wheat flour, 1.13 μg/g; whole wheat bread, 1.55 μg/g; dried yeast, 65.7 μg/g. The other applied two microbiological and two fluorometric methods to the assay of the sample of whole wheat flour. The committee's methods yielded 0.87 μg/g microbiologically and 1.24 μg/g fluorometrically. The collaborator's own methods gave 0.92 μg/g and 0.97 μg/g, respectively, for the two types of procedures.

Literature cited

Andrews, J. S., Boyd, H. M., and Terry, D. E.
1942 The riboflavin content of cereal grains and bread and its distribution in products of wheat milling. Cer. Chem. **19**: 55–64.

Andrews, J. S., Boyd, H. M., and Terry, D. E.
1942 Riboflavin analysis of cereals. Application of the microbiological method. Ind. Eng. Chem. (Anal. Ed.) **14**: 271–274.
Bauernfeind, J. C., Sotier, A. L., and Boruff, C. S.
1942 Growth stimulants in the microbiological assays of riboflavin and pantothenic acid. Ind. Eng. Chem. (Anal. Ed.) **14**: 666–671.
Conner, R. T., and Straub, G. J.
1941 Combined determination of riboflavin and thiamin in food products. Ind. Eng. Chem. (Anal. Ed.) **13**: 385–388.
Hodson, A. Z., and Norris, L. C.
1939 A fluorometric method for determining the riboflavin content of foodstuffs. J. Biol. Chem. **131**: 621–630.
Kemmerer, A. R.
1941 Report on riboflavin. Jour. of A.O.A.C. **24**: 413–423.
Scott, M. L., Randall, F. E., and Hessel, F. H.
1941 A modification of the Snell and Strong microbiological method for determining riboflavin. J. Biol. Chem. **141**: 325–326.
Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) **11**: 346–350.

SOYBEAN AMYLASE. II. FURTHER PURIFICATION OF CRUDE SOYBEAN BETA-AMYLASE CONCENTRATES[1]

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In an earlier paper (Newton and Naylor, 1939), the preparation of beta-amylase concentrates from soybeans was described. Concentrates so prepared have been utilized in studies of the comparative rates of amylase action on starches (Martin and Newton, 1938), in the preparation of the products of the digestion of starch by beta-amylase (Martin, 1938; Martin, Naylor, and Hixon, 1939), to follow the modification of starch (Newton, Farley, and Naylor, 1939), and in studies on the fractionation of starch (Caldwell, 1941). These applications of the soybean amylase preparation demonstrate the uses of the crude beta-amylase concentrates and indicate the value of further purification. This paper reports studies on further purification and the properties of the highly concentrated preparations.

Preparation of Crude Beta-Amylase Concentrates

The method of Newton and Naylor (1939), with slight modifications, was utilized. The method involves the precipitation of amylase concentrates from water extracts with alcohol. Petroleum ether (bp 68°–70°C) was substituted for diethyl ether in extraction of the

[1] Supported in part by a grant from the Industrial Science Research Institute funds of Iowa State College for the study of soybean amylase.
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oil from the ground beans. Table I presents the results of several typical preparations. Three general statements apply to these amylase concentrates: (1) With increased alcohol concentration the yield of precipitate increased. (2) The saccharogenic power of the amylase concentrates usually varied inversely with the yield of the precipitate. (3) Those concentrates precipitated in greater yields and at higher alcohol concentrations were more soluble in water than the concentrates obtained in lower yields and at lower alcohol concentration.

TABLE I

YIELD FIGURES FOR TWELVE CRUDE AMYLASE PREPARATIONS

Preparation No.	Extracted bean used	Concentrate obtained	Alcoholic concentration for precipitation	Saccharogenic power[1]	Amylase units[2]	Yield[3]
	g	g	%			%
1	500	0.72	65	745	536	5.4
2	220	1.05	65	990	1040	23.6
3	480	2.45	70	1000	2450	24.5
4	500	2.28	70	1209	2750	27.5
5	500	2.80	70	653	1700	17.0
6	500	3.91	70	512	2000	20.0
7	500	12.45	70	219	2730	27.3
8	600	2.13	70	1072	—	—
9	600	4.24	70	528	—	—
10	600	3.74	70	455	—	—
11	600	4.25	70	399	—	—
12	600	1.20	68	753	—	—

[1] Saccharogenic power is defined as the milligrams of maltose produced per milligram of amylase concentrate under the conditions specified for the determination of beta-amylase activity (Newton and Naylor, 1939).

[2] Amylase units are obtained by multiplying the saccharogenic power of the concentrate by the yield in grams.

[3] The saccharogenic power of the extracted beans was 20.

$$\text{Yield in percent} = \frac{(\text{g extracted bean used})\ (20)\ (100)}{(\text{g concentrate obtained})\ (\text{saccharogenic power of concentrate})}$$

Further Purification of Crude Beta-Amylase Concentrates

Little or no success was obtained in attempts to further purify the crude beta-amylase concentrates when alcohol was used as the precipitating agent. Dilute alcohol at room temperature and higher alcohol concentrations at lower temperatures caused a marked decrease in the activity of the amylase concentrates. This observation is in agreement with the quantitative measurement of the effect of alcohol and temperature on the amylase of wheat as described by Blish, Sandstedt, and Mecham (1937). By the substitution of acetone for alcohol and by using as low temperature as convenient, considerable purification was obtained. Even then the recovery of the active enzyme and the degree of purification were not as great as desired. Improvement in the concentration procedure was observed when the

drying of concentrates was eliminated until the purification was complete. Purification was further increased by the incorporation of electrodialysis into the procedure (for description of electrodialyzer see Hixon and Martin, 1939). This process removed considerable quantities of organic impurities as well as inorganic salts.

Therefore, in attempts to purify crude beta-amylase concentrates further, acetone was substituted for ethyl alcohol, temperatures were maintained at as low a level as possible, precipitates were not dried until purification was complete, and electrodialysis was used to remove organic impurities and inorganic salts. The flow sheet for a typical preparation is given in Table II. The yields of beta-amylase by the

TABLE II

FLOW SHEET FOR THE PREPARATION OF BETA-AMYLASE CONCENTRATES FROM SOYBEANS

Moist crude beta-amylase concentrate from 1000 g hexane extracted soybeans (saccharogenic activity of beans = 23.1). Extracted with three 80-ml portions of water ..... Residue I

↓

Amylase solution (250 ml) electrodialyzed 1.8 hours ..... Ppt. II

↓

Amylase solution. Acetone to 50% at −5°C ..... Precipitate separated by centrifugation but not dried.

↓

Acetone to 60% at −5°C gave Ppt. III

↓

Acetone to 75% at −5°C gave Ppt. IV

↓

Solution evaporated to dryness. Residue V

↓

Extracted six times with 20-ml portions 40% acetone. Residue VI.

↓

Solution evaporated to 20.5 ml at 25°C. Acetone to 50% at −5°C gave Ppt. VII.

↓

Acetone to 60% at −5°C gave Ppt. VIII.

↓

Acetone to 80% at −5°C gave Ppt. IX.

↓

Solution evaporated to dryness. Residue X.

concentration procedure given in Table II are presented in Table III. As in Table I, the yield of enzyme is based on the amylase units recovered in the various fractions and these results are shown in the last column of Table III.

In the particular experiment for which the data are presented in Table III the total yield of amylase was 10.7%. This was somewhat lower than the percentage yields given in Table I for a series of crude soybean amylase concentrates. The lower yield in the experiment presented in Table II and Table III was due to several causes, the

exact contribution of each cause being undeterminable. Some of the contributing causes were mechanical loss in the manipulation of small quantities of the highly active concentrates; loss due to slight inactivation during electrodialysis, fractional solution, and fractional precipitation; inactivation by the organic solvents; and the spontaneous inactivation of the amylase solutions which occurred during the long concentration procedure.

TABLE III

TOTAL YIELD OF BETA-AMYLASE CONCENTRATES BY THE PROCEDURE GIVEN IN TABLE II

Precipitate number	Yield in grams	Saccharogenic activity	Amylase units
I	0.4205	0	0
II	0.7580	0	0
III	0.1883	—	—
IV	7.3390	232	1702.9
V	3.3065	0	0
VI	0.2021	251	50.7
VII	0.1066	2260	240.9
VIII	0.0661	4580	302.7
IX	0.0815	2155	175.6
X	0.0837	0	0
Total yield in amylase units			2472.8
Amylase units in 1000 grams extracted beans			23,100
Yield of amylase (as %)			10.7

From Table III it is evident that a large portion of the total amylase recovered remains in precipitate IV which has an activity of only 232. An attempt was therefore made to concentrate this material further and to follow the yields of the enzyme. Different procedures based on fractional solution and fractional precipitation with acetone-water mixtures were employed. The best results were obtained by dissolving one gram of precipitate IV (Table III) in 30 ml of 50% acetone at room temperature. Complete solution required several minutes of continuous stirring. Upon cooling in an ice bath a small amount of solid material precipitated. This was collected by centrifugation, dried and preserved as precipitate A. The acetone concentration of the solution at 0°C was raised to 53.5% to give precipitate B, to 65.5% to give precipitate C, and to 75% to give precipitate D. The centrifugate from D was then evaporated at room temperature to give residue E. The recovery of solids and the activities of the various fractions are presented in Table IV. These data show a 5.5- and 10-fold concentration of the beta-amylase in precipitates A and B, respectively, with a 68% recovery of the total amylase.

The recovery of amylase by this method was larger than shown by other data in the literature. For example Sherman and Neun (1919)

in studies on the purification of amylase were able to recover 13.5, 19.76, 24.83, 24.95, and 27.81% of the amylase in five separate experiments. This compares with 60.8, 63.2, and 68.0% in three experiments with soybean beta-amylase concentrates.

TABLE IV

PRODUCTS OBTAINED FROM THE PURIFICATION OF ONE GRAM OF AN AMYLASE CONCENTRATE WITH SACCHAROGENIC ACTIVITY OF 232

Precipitate number	Recovery of solids	Saccharogenic activity	Amylase units
	g		
A	0.0184	1248	25.0
B	0.0383	2310	88.5
C	0.2987	148	44.2
D	0.2635	0	—
E	0.2188	0	—
Totals	0.8377		157.7
Amylase units in original starting material			232.0
Recovery (%)	83.8		68.0

Deterioration and Inactivation of Soybean Beta-Amylase

Several dry beta-amylase concentrates were combined to give 16 g of material. The concentrate was stored in small screw-top vials containing about 2 g of the enzyme concentrate in each. At the time of combination the saccharogenic activity was 586, after five months it was 600, and after two years' storage it remained at 600. This demonstrates the stability of the dry concentrate when stored in the ordinary refrigerator.

Even at higher temperatures the enzyme is quite stable. Heating the ground soybeans for 10 days at 100°C did not completely inactivate the amylase. On the other hand, soybeans roasted commercially for sale as a confection contained no traces of an active amylase. In another experiment a portion of the soybean amylase concentrate was heated at 200°C for 30 minutes. This treatment completely destroyed the amylase. Thus the amylase is destroyed by dry heat at higher temperatures.

The combined effect of organic solvent and heat was also investigated. A sample of soybeans which had been extracted in a continuous extractor on a pilot-plant scale using trichloroethylene as a solvent, with the excess solvent being removed from the beans by heating at 100–130°C for 60 minutes, was tested for amylolytic activity. About 70% of the activity was destroyed. Another sample of soybeans, extracted commercially with hexane, had been heated at 95°C for 30 minutes to remove most of the solvent and then with superheated steam to remove the last traces of the hexane. Tests on

this material showed complete destruction of the amylase. A sample of beans taken from this extraction process before exposure to the super-heated steam showed no marked decrease in activity. These experiments show that soybean beta-amylase is resistant to the action of organic solvents. However, if in removing these solvents temperatures above 100°C (in the presence of moisture) are used or if the exposure at 100°C continues over too long a period of time, the amylase is inactivated.

Numerous reports in the literature (Sherman and Tanberg, 1916; Sherman and Schlesinger, 1912, 1915; Ono, 1936; Ohlsson, 1926) show the marked rate of inactivation of aqueous solutions of amylase when held at or slightly above room temperature. In fact, the Ohlsson method (1926) for the preparation of alpha-amylase concentrates is dependent on the differential inactivation of beta-amylase by heating at 70°C. In this procedure the beta-amylase of barley is destroyed by heating an aqueous solution of alpha- and beta-amylase at pH 6–7 for 10 minutes at 70°C. The same method was used (Creighton and Naylor, 1933) for the preparation of purified beta-amylase from wheat. That the beta-amylase of soybeans is not destroyed at this temperature is demonstrated by the fact that digestions of starch paste with this enzyme can be carried on at 76°–78°C. For example, 10 g of cornstarch and 100 ml of water were heated to 78°C in a water bath. A warm-water suspension of soybean amylase was added, mixed thoroughly and allowed to digest for 30 minutes at 76°–78°C. At the end of the digestion period the digest was heated to boiling, diluted, and reducing sugars determined on an aliquot. The determined maltose equivalent of 2.28 g represented over 70% of the maltose equivalent obtainable if the quantity of enzyme present could have acted on the starch under ideal conditions (40°C in 2% starch paste at pH 5.0).

The rate of deterioration of soybean beta-amylase concentrate in aqueous solution was also investigated. Other investigations (Sherman and Schlesinger, 1915; Ono, 1936) have shown that the most highly purified concentrates deteriorate most rapidly. This was not observed with soybean beta-amylase. Samples of six of the most active concentrates prepared were dissolved in distilled water and allowed to stand at room temperature. Figure 1 presents the results of this study. The most highly purified concentrates used in preparing solutions VIII, IX, and X retained 79%, 76%, and 62% of their respective activities after standing 34 days at room temperature. On the other hand the three less highly purified concentrates, I, III, and VII, were inactive after 30 days. The reason for the greater rate of deterioration of the least highly purified concentrates is not definitely known. The small variations in the method of preparation of the

concentrates do not account for the observed differences. A more satisfactory explanation probably lies in the degree of purification, the more highly purified concentrates containing smaller quantities of

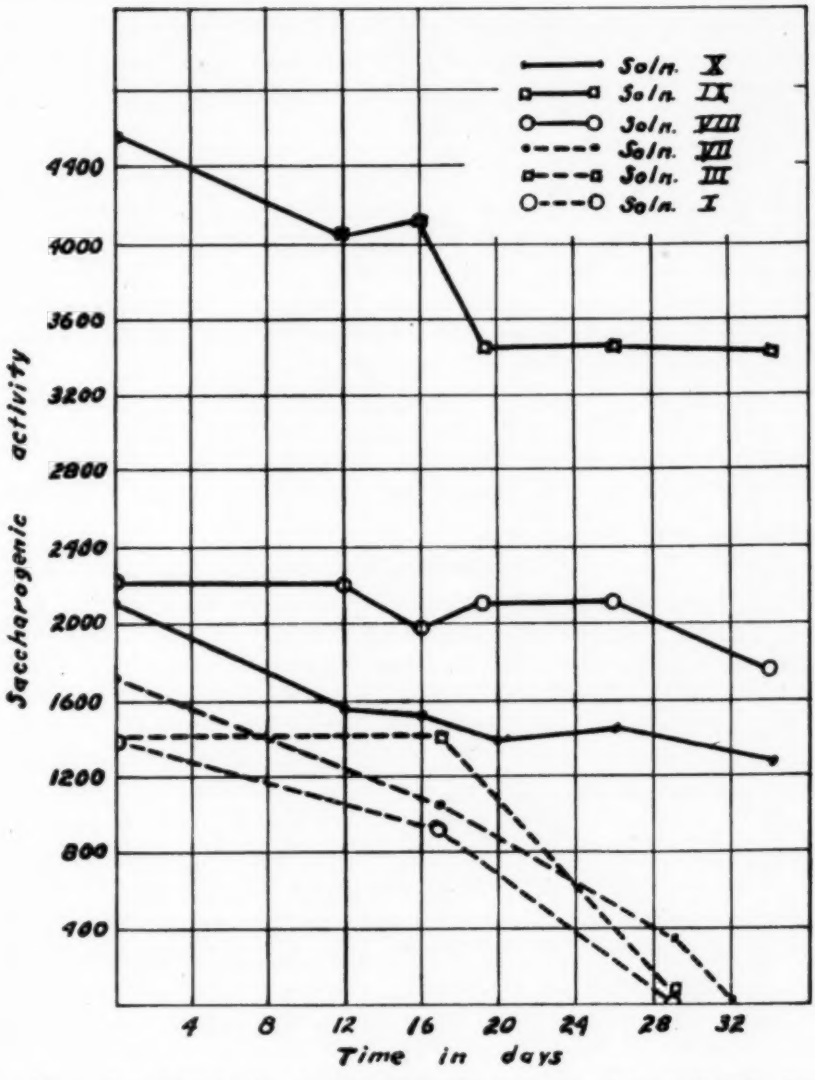

Fig. 1. Rate of deterioration of soybean beta-amylase in aqueous solution at 25–30°C. Concentration of solutions: I, III—40 mg in 50 ml; VII—9.2 mg in 10 ml; VIII—16.4 mg in 50 ml; IX—16.3 mg in 50 ml; X—15.6 mg in 50 ml.

substances that are subject to bacterial action, this bacterial action either destroying the enzyme or producing substances which inactivate the enzyme. This explanation is suggested by the observation that

after standing for 8–10 days a marked putrefactive odor existed in all solutions except sample IX. At no stage was any marked odor observed in this solution. At the end of two weeks a considerable growth of organisms was evident in solutions I, III, and VII, a slight but distinct cloudiness was observed in VIII and X, while solution IX remained water clear throughout the experiment. A preservative was not used in these solutions because of the possible deleterious action on the enzyme.

Summary

In the several preparations of soybean beta-amylase described the dry concentrate contained from 17% to 27% of the extractable beta-amylase present in the original soybeans.

A method is given for further purification of the crude soybean beta-amylase concentrates. The most concentrated preparation produced 4,580 times its weight of maltose in 30 minutes at 40°C.

The concentration procedure has been applied to low-activity materials with satisfactory concentration and recovery of the beta-amylase.

The deterioration and inactivation of soybean beta-amylase has been investigated.

Literature Cited

Blish, M. J., Sandstedt, R. M., and Mecham, D. K.
1937 Action of wheat amylases on raw wheat starch. Cereal Chem. **14**: 605–628.

Caldwell, C. G.
1940 Fractionation of starch. Doctoral dissertation. Iowa State College Library.

—— and Hixon, R. M.
1941 Fractionation of waxy and ordinary cornstarch. J. Am. Chem. Soc. **63**: 2876.

Creighton, Mattie, and Naylor, N. M.
1933 A study of the starch-digestion and sugar-forming enzymes of wheat. Iowa State College J. Sci. **7**: 253.

Hixon, R. M., and Martin, Vera Dawson.
1939 An electrodialyzer for starch. Ind. Eng. Chem. (Anal. Ed.) **11**: 395.

Martin, V. D.
1938 Characterization of some products of starch-enzyme digestion. Thesis. Iowa State College Library.
1938 Characterization of certain products of starch-enzyme digestion. Iowa State College J. Sci. **13**: 81–83.

—— Naylor, N. M., and Hixon, R. M.
1939 Action of beta-amylase from soybeans on various starches. Cereal Chem. **16**: 565–662.

—— and Newton, J. M.
1938 Comparative rates of amylase action on starches. Cereal Chem. **15**: 456–462.

Newton, J. M., Farley, F. F., and Naylor, N. M.
1939 The use of soybean beta-amylase to follow the modification of starch. Cereal Chem. **17**: 342–355.

—— and Naylor, N. M.
1939 Soybean amylase. I. The concentration and characterization of soybean amylase. Cereal Chem. **16**: 71–78.

Ohlsson, E.
1926 On the two components of malt diastase. Compt. rend. trav. lab. Carlsberg **16** (No. 7): 1–68.
Ono, K.
1936 Yeast amylase. VI. Thermostability of yeast amylase. J. Agr. Chem. Soc. Japan **12**: 191–195; Chem. Abst. **30**: 6771.
Sherman, H. C., and Neun, D. E.
1919 Proteolytic activity of pancreatic amylase preparations. J. Am. Chem. Soc. **41**: 1855–1862.
—— and Schlesinger, M. D.
1912 Studies on amylases. IV. A further investigation of the properties of pancreatic amylase. J. Am. Chem. Soc. **34**: 1104–1111.
1915 Studies on amylases. X. Comparison of certain properties of pancreatic and malt amylase preparations. J. Am. Chem. Soc. **37**: 1305–1319.
—— and Tanberg, A. P.
1916 Experiments upon the amylase of *Aspergillus oryzae*. J. Am. Chem. Soc. **38**: 1638–1645.

A COLLABORATIVE STUDY OF METHODS FOR THE DETERMINATION OF AMYLASE ACTIVITY[1]

ALLAN D. DICKSON, *Chairman*

Division of Cereal Crops and Diseases, U. S. Department of Agriculture[2]

(Read at the Annual Meeting, May 1942)

Last year's Committee undertook, as a part of its activities, a study of methods for the determination of alpha-amylase. The results of this study were rather unsatisfactory since only three laboratories submitted data, and one differed from the others in extraction procedure. Fairly good agreement was obtained between two laboratories with the Sandstedt, Kneen, and Blish (1939) dextrinizing procedure and with the Józsa and Johnston (1935) viscometric method. The agreement with the Blom and Bak (1938) method was unsatisfactory. Within laboratories the correlation of data obtained by the three methods on the six malts was very high, indicating that all methods were measuring the same thing.

In a joint collaborative study with the Subcommittee on Diastatic Power of the American Society of Brewing Chemists, the Committee this year undertook a study of available methods for the determination of specific components of the amylase system of malts.

Experimental

Six samples of malt, selected for their range in diastatic power, were submitted to 18 laboratories. All laboratories were requested to determine diastatic power by either the Fehling's titration or ferricyanide modifications. Other methods studied by some of the collab-

[1] Report of Malt Analysis Standardization Committee (1941–1942).
[2] Stationed at Madison ,Wisconsin.

orators were: (1) Kneen and Sandstedt (1941) measure of saccharification, (2) Sandstedt, Kneen, and Blish (1939) method for alpha-amylase by a dextrinization procedure, and (3) Blom and Bak's (1938) and (4) Józsa and Johnston's (1935) viscometric methods for alpha-amylase.

The two methods from the Nebraska Station were used as published, except that extraction of the malt was carried out with 5 g per 100 ml for 1 hour at 30°C instead of 1 g per 100 ml. In the dextrinization procedure a uniform sample of beta-amylase, kindly supplied by Wallerstein Laboratories, was used in the preparation of the substrate instead of the recommended hard wheat flour extract.

Presentation and Discussion of Data

The submitting of samples was delayed by the difficulty of obtaining a satisfactory source of beta-amylase. Therefore most of the data submitted were on diastatic power and on the Nebraska methods, which require somewhat less standardization than the viscometric

TABLE I

COLLABORATIVE DATA ON DIASTATIC POWER

Collaborator No.	Malt 1		Malt 2		Malt 3		Malt 4		Malt 5		Malt 6	
	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose
1[1]	78.1	313	116.6	467	140.3	561	155.1	620	182.1	729	191.8	768
2[2]	78.0	312	108.0	432	122.0	488	142.0	568	162.0	648	172.0	688
3[1]	75.7	303	102.8	411	127.5	510	148.8	594	164.8	659	174.9	700
4[1]	82.9	332	109.0	436	132.0	528	152.0	608	173.5	694	182.0	728
5[1]	80.0	320	106.0	424	128.5	514	154.0	616	166.0	664	181.5	726
6[1]	79.5	318	110.5	442	135.0	540	150.5	602	172.0	688	186.5	746
7[1]	86.3	345	118.0	472	146.4	586	165.0	660	191.0	764	197.7	791
8[1]	75.6	302	104.4	418	127.8	511	144.9	580	161.1	645	171.9	688
9[1]	78.7	315	109.6	439	134.5	538	152.2	609	168.1	673	182.2	729
10[1]	81.0	324	113.0	452	137.0	548	153.0	612	178.0	712	183.5	734
11[1]	81.7	327	114.5	458	136.6	546	150.5	602	168.7	675	180.7	723
12[2]	84.0	336	110.0	440	131.0	524	151.0	604	166.0	664	179.0	716
13[2]	83.3	333	92.2	369	124.3	497	138.0	552	165.0	660	173.9	696
15[2]	86.0	344	116.0	464	137.0	548	154.0	616	168.0	672	182.0	728
16[2]	79.1	316	110.2	441	134.5	538	149.0	596	179.1	716	191.3	765
17[2]	82.0	328	121.0	484	141.0	564	158.0	632	171.0	684	189.0	756
M[3]	80.7	323	110.1	441	133.0	534	151.1	604	171.0	684	182.5	730
SDbD[4]	1.66	—	1.47	—	1.49	—	2.71	—	2.60	—	1.67	—
CVbD[5]	2.06	—	1.34	—	1.12	—	1.82	—	1.52	—	0.92	—
SDbL[6]	3.13	—	6.26	—	6.14	—	5.96	—	8.05	—	7.21	—
CVbL[7]	3.88	—	5.72	—	4.62	—	3.96	—	4.71	—	3.95	—

[1] Ferricyanide modification used for diastatic power.
[2] Fehling's modification used for diastatic power.
[3] Mean.
[4] Standard deviation between duplicates.
[5] Coefficient of variability between duplicates.
[6] Standard deviation between laboratories.
[7] Coefficient of variability between laboratories.

methods. The results obtained with the diastatic power are presented in Table I. The Kneen and Sandstedt saccharification activities and calculated beta-amylase values are presented in Table II. Means of duplicate determinations, standard deviations, and coefficients of variability between duplicates and between laboratories are also given.

TABLE II

COLLABORATIVE DATA ON SACCHARIFICATION ACTIVITY AND BETA-AMYLASE UNITS BY THE KNEEN AND SANDSTEDT METHOD

Collaborator No.	Malt 1		Malt 2		Malt 3		Malt 4		Malt 5		Malt 6	
	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units
1	133.1	8.7	101.3	12.5	116.8	14.8	115.8	15.2	135.1	18.4	151.3	19.6
2	135.6	8.5	94.5	11.9	102.8	12.9	112.4	14.4	134.9	18.0	147.8	18.7
3	114.5	7.2	74.6	8.8	98.3	12.1	106.8	13.8	119.8	15.9	143.2	18.2
4	127.0	8.1	84.8	10.6	103.5	13.1	117.5	15.3	135.0	18.1	145.5	18.3
5	129.6	8.2	89.1	11.0	113.7	14.2	130.4	16.9	138.6	18.5	150.1	18.4
6	129.3	8.7	89.3	11.8	111.8	14.7	125.5	16.7	127.1	17.2	137.0	18.0
7	126.5	7.9	88.1	10.9	114.5	14.3	123.2	16.0	136.7	18.3	150.5	18.5
8	127.3	8.0	83.9	10.0	100.6	12.4	109.0	13.9	121.6	16.1	142.7	17.7
9	127.1	8.4	95.2	12.4	114.2	15.0	131.3	17.8	139.6	19.1	153.5	20.1
12	139.6	9.1	88.2	11.1	110.9	14.1	126.5	16.6	143.9	19.3	150.2	19.0
15	131.2	—	93.3	—	110.9	—	126.2	—	145.3	—	154.5	—
17	130.2	—	90.8	—	115.0	—	127.0	—	136.0	—	164.5	—
M[1]	129.2	8.3	89.4	11.1	109.4	13.8	121.0	15.7	134.5	17.9	149.2	18.7
SDbD[2]	3.21	0.23	3.17	0.49	3.84	0.59	2.73	0.35	3.94	0.60	4.34	0.67
CVbD[3]	2.48	2.77	3.55	4.41	3.53	4.28	2.27	2.23	2.93	3.35	2.94	3.58
SDbL[4]	6.07	.50	6.67	1.08	6.10	0.99	8.16	1.29	7.92	1.09	5.02	0.70
CVbL[5]	4.70	6.05	7.47	9.73	5.60	7.17	6.78	8.22	5.90	6.09	3.40	3.74
DP °L/mg maltose/50 ml	1.24	—	1.24	—	1.23	—	1.27	—	1.29	—	1.24	—

[1] Mean.
[2] Standard deviation between duplicates.
[3] Coefficient of variability between duplicates.
[4] Standard deviation between laboratories.
[5] Coefficient of variability between laboratories.

The data submitted on the three alpha-amylase methods are given in Table III. The statistics were calculated only for the dextrinizing method.

Considering diastatic power and the Nebraska methods, the variability between laboratories is greatest for the alpha-dextrinizing method, although the variation between duplicates is of about the same order as for the saccharification method. This might easily have been caused by lack of standardization in the procedure of making color comparisons in different laboratories. The opinion was expressed by several collaborators that the variation in both of the Nebraska methods, which was greater than with the diastatic power method, could be reduced by extracting a larger quantity of malt. This modification might profitably be applied to barley malts, where the quantity of hull material is high. From the data it appears that the Nebraska methods warrant further study, with perhaps certain modifications and greater standardization of procedure. This is especially desirable

TABLE III

COLLABORATIVE DATA ON THREE METHODS FOR THE DETERMINATION OF ALPHA-AMYLASE

Collaborator No.	Malt 1	Malt 2	Malt 3	Malt 4	Malt 5	Malt 6
	Alpha-amylase units—Sandstedt, Kneen, and Blish dextrinizing method					
1	29.0	49.3	58.3	48.1	42.0	68.6
2	29.1	49.9	54.2	54.1	50.5	76.8
3	31.0	50.0	57.5	49.9	46.9	71.1
4	31.3	45.2	53.0	51.9	47.2	78.4
5	33.1	50.6	61.9	59.1	50.6	89.3
6	21.8	35.6	45.8	46.9	42.7	56.5
7	35.0	49.9	61.9	55.7	50.5	89.4
8	33.1	54.9	58.2	54.1	46.9	80.0
9	24.3	40.7	47.3	44.0	41.5	68.6
12	31.0	47.0	55.0	53.0	52.0	78.0
14	23.0	36.7	44.0	43.6	40.6	61.3
M[1]	29.2	46.3	54.3	50.9	46.5	74.4
SDbD[2]	0.94	1.62	2.36	1.90	1.97	2.03
CVbD[3]	3.22	3.50	4.35	3.73	4.24	2.73
SDbL[4]	4.18	5.88	5.93	4.69	3.98	9.82
CVbL[5]	14.32	12.70	10.92	9.21	8.56	13.20
	Alpha-amylase units—Blom and Bak method					
1[6]	61.0	79.0	113.0	111.0	93.0	137.0
9[6]	72.4	103.8	129.1	124.1	124.6	177.6
14[6]	76.2	121.5	137.5	133.5	128.0	188.0
1[7]	34.1	44.1	63.1	62.0	52.0	76.5
4[8]	33.3	48.9	54.7	46.2	51.4	81.0
9[8]	40.6	61.9	72.3	68.4	65.9	98.9
12[8]	42.2	60.5	79.9	72.2	72.4	115.2
14[7]	42.6	67.9	76.8	74.6	71.5	105.0
	Alpha-amylase units, liquefons per gram—Józsa and Johnston method					
4	236	362	453	416	393	570
14	221	394	415	413	356	676

[1] Mean.
[2] Standard deviation between duplicates.
[3] Coefficient of variability between duplicates.
[4] Standard deviation between laboratories.
[5] Coefficient of variability between laboratories.
[6] Viscometer standardized against 45% by weight of sucrose solution.
[7] Data from Collaborators 1 and 14 adjusted by ratio of 1.79 obtained from data of Collaborator 9 using both procedures.
[8] Viscometer standardized against 45% by volume (45 g per 100 ml) of sucrose solution.

since these methods are more suited to routine application than the viscometric methods. The ratios of diastatic power to malt saccharification values on the six malts indicate the same close relationship between these two factors, as shown by Kneen, Beckord, and Sandstedt (1941).

The limited amount of data on the two viscometric methods was not sufficient for a good evaluation. As a result of an error in translation of the original Blom and Bak method, different-strength sucrose solutions were used for standardization of the viscometer. This resulted in values of different magnitude. By using viscometers standardized against sucrose solutions of 45% by weight and 45% by volume (45 g per 100 ml) for the determinations on the six malts, a relatively constant ratio which averaged 1.79 was found between the values obtained with the two viscometers. The data of Collaborators No. 1 and No. 14 were obtained with viscometers standardized against 45%-by-weight sucrose solution. These values were calculated to the other standardization using the above ratio and are given in Table II. The variation between laboratories is still much too high and further study of this method would be desirable.

Only two laboratories reported results obtained with the Józsa and Johnston method. The agreement is fairly good for most of the malts. It is evident that the viscometric procedures give reliable results only in the hands of experienced operators, and even there the variation is too great. A comparison of these procedures in a smaller number of laboratories which are experienced in their use and on a larger number of samples would be a better method of evaluation.

In view of the above data the committee recommends that the various methods for alpha- and beta-amylases be submitted to further study, with the purpose of determining the cause of variability in results and of modifying the procedures to give better agreement between laboratories.

Acknowledgment

The chairman expresses his appreciation to members of the committee, to the collaborators, whose willing cooperation under trying conditions made this report possible, and to B. A. Burkhart and W. J. Olson of this laboratory for their assistance.

Literature Cited

Blom, J., and Bak, A.
1938 Über die Bestimmung von Amylasen durch Verflussigung von Stärkekleister. Z. physiol. Chem. **256**: 197–207.
Józsa, S., and Johnston, W. R.
1935 Determination of alpha-amylase. Ind Eng. Chem. (Anal. Ed.) **7**: 143–146.
Kneen, E., and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18**: 237–252.
Kneen, E., Beckord, O. C., and Sandstedt, R. M.
1941 The starch degrading properties of barley malts. Cereal Chem. **18**: 741–754.
Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.

REPORT OF METHODS COMMITTEE ON THE DETERMINATION OF IRON IN CEREAL PRODUCTS

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(Read at the Annual Meeting, May 1942)

Five samples each of unenriched flour, enriched flour, bread, and whole wheat were sent to eleven laboratories for collaborative work on iron determination. Eight laboratories replied, and the results are summarized in Table I.

Complete details of a method were outlined in the directions sent to collaborators. This method was essentially that outlined by Andrews and Felt (1941). The regular ashing of the samples at 550°–600°C plus the use of hydrochloric acid for the solution of the ash was specified, as was also the use of platinum dishes. Porcelain dishes have proved equally satisfactory.

Sample No. 2, which was Sample No. 1, a short patent, enriched with 6 mg of ferrum reductum per pound of flour, is not included in

TABLE I

DETERMINATION OF IRON CONTENT IN CEREAL PRODUCTS

(Moisture on the "as is" basis)

Collaborator No.	Sample 1—unenriched short patent flour	Sample 3—No. 1 enriched with 6 mg per lb Fe as sodium iron pyrophosphate	Sample 4[1]—bread from No. 1 enriched with 6 mg per lb of ferrum reductum	Sample 5—whole wheat, fine granulation
	mg/lb	*mg/lb*	*mg/lb*	*mg/lb*
1	3.3	9.1	11.3	19.9
2	3.3	9.1	11.5	19.4
3	3.7	9.1	11.4	21.5
4	3.7	9.0	12.0	29.0*
5	2.6	7.2*	10.0	15.8*
6—Ashing at				
1350°F	3.5	10.2*	17.0*	19.4
1100°F	2.3*	9.4*	15.3*	19.2*
7—Regular ashing	3.4	3.6*	9.1*	14.5*
Alkaline ashing (NaOH)	3.4*	14.1*	14.2*	20.4*
8—Regular ashing	3.8	9.1	11.1	17.2
Sodium carbonate fusion	4.1*	10.8*	14.2*	21.4*
Average	3.4	9.1	11.2	19.5

* Starred results were omitted from the averages. They were either too far from the average results obtained on the alkaline treatment used in some laboratories (Collaborators No. 7 and No. 8) or were duplicates done in a different manner (No. 6). Results by the alkaline treatment of the sample were also omitted merely to keep the variables at a minimum.

[1] Bread was made from a straight dough with 2% yeast, 2% salt, 5% sugar, 3% dry skim milk, 3% shortening, and 0.25% yeast food. All results were higher than the theoretical based on the iron content of flour owing probably to additional iron contributed by other ingredients such as the yeast food.

the summary. Directions were sent to each collaborator asking that this sample be sifted through a wire screen before analysis. Since some collaborators did not follow these directions, it was felt that this flour should be excluded from the report, although the range in iron content found was no greater than in the other samples. Every effort was made by thorough mixing and repeated sifting of samples to insure uniformity and equal distribution of iron in cases where iron was added.

From our own experience as well as from the comments and results of individual collaborators the main difficulty seemed to be in the preparation of the samples for analysis and in the ashing and solution of the ash rather than in the determination itself.

Two collaborators reported significantly higher results on alkaline fusion with NaOH or Na_2CO_3. In the instance of one collaborator using alkaline fusion as well as the regular ashing in platinum, sulfocyanate was used to develop the color rather than α, α'-dipyridyl. This collaborator reported results significantly higher on alkaline ashing than by the regular procedure on all except Sample No. 1. Another collaborator reported results by the procedure outlined as well as with the use of *o*-phenanthroline for color development. This laboratory also reported results with a sodium carbonate ashing in silica dishes. Results by the latter method were significantly higher and also higher than the average figures.

One laboratory reported as follows: "Due to the burning out of the rheostat on the furnace the first set of samples was ashed at 1350°F (730°C) rather than at 1100°F (593°C). Ashing at 1350°F gave consistently higher results than ashing at the lower temperature of 1100°F."

Other than the above departures, all other chemists followed the method as outlined. So far as we are aware, all laboratories used some type of electrophotometer for color comparison.

Results were disappointing and it is felt that most of the discrepancies arise from the preparation of the samples for analysis.

Before there was such general interest in the iron content of flours, our own laboratory had run many determinations on wheats and milling separations. We were never able to find any loss of iron under the conditions used in ashing. Samples were always ashed in an electric muffle at 600°C overnight and the ash dissolved in acid (either nitric or hydrochloric). In the paper previously cited, Andrews and Felt (1941) state that in using overnight ashing at 575°C there is no evidence of any loss of iron in flour or breads.

After the results reported in this collaborative work were received, we checked a sample of flour with added iron prepared by direct

ashing as well as the same sample prepared by Na_2CO_3 treatment of the flour prior to ashing. Identical results were obtained by both methods.

On the other hand, Hoffman, Schweitzer, and Dalby (1940), on the basis of their work on cereal products, believe that an alkaline ashing procedure is essential to secure complete recovety of added iron.

The agreement between results of collaborators is unsatisfactory. Since samples were sent only to laboratories having excellent reputations for analytical work, there must be some condition in ashing or in the solution of the sample in hydrochloric acid which is encountered in some laboratories and of which we are not aware. A few points in this regard may be mentioned. Porcelain dishes have proved equally as satisfactory as platinum. It is imperative that dishes used for ashing in the preparation of the sample for iron determination not be used for any other purpose during the time such determinations are being made. When, on occasion, we have used our platinum crucibles for ashing mixed feeds, for instance, the flour or bread samples ashed in these dishes gave erratic results even though before use the crucibles had been thoroughly cleaned with boiling HCl. In order that all the iron go in solution from the ash, boiling in HCl should be continued a few minutes with special precautions taken to see that no spattering occurs. Incomplete solution of the iron in the ash and loss by spraying during boiling are frequent reasons for low results.

The results have not been statistically analyzed since it was felt that the number of collaborators was too small. Only the averages have been shown. It is apparent that further collaborative work should be done on checking the iron content of cereal products since there are some still obscure reasons why more concordant results have not been obtained among laboratories. This is true even of unenriched short-patent flour and whole-wheat flour to which nothing was added.

It is recommended that collaborative work on the measurement of iron be continued with particular emphasis on the ashing and preparation of the sample solution. It would be advisable to send with the flours or bread a solution of known iron content.

Literature Cited

Andrews, J. S., and Felt, C.
1941 The iron content of cereals. Cereal Chem. **18**: 819–827.
Hoffman, C., Schweitzer, T. R., and Dalby, G.
1940 Iron content of bread and bread ingredients. Ind. Eng. Chem. (Anal. Ed.) **12**: 454–455.

DESCRIPTION OF MIXOGRAMS[1]

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With the increasing use of the National Swanson-Working recording dough mixer in the study of wheat and flour quality problems, a need exists for a simple diagram which may be used both in describing curve characteristics and in correlating these characteristics with baking and other quality measurements. This paper deals with proposed descriptive diagrams. The correlations with other quality measurements will follow in future papers.

A diagram that gave the meaning of the characteristics of curves obtained on the recording dough mixer was presented by Swanson and Clark (1936) together with typical curves obtained from flours of well-known wheats. The same diagram with curves was also shown by Swanson (1937). These curves were obtained on the first model of the recording dough mixer (Swanson and Working, 1933). The curves made on that first model differ from those obtained on the National Swanson-Working mixer mainly in mixing time, since the rate of movement of the chart paper of the former machine is only one-third that of the latter. Curves made on the first model of the Swanson-Working micro-mixer were presented by Larmour, Working, and Ofelt (1939). A simple diagram was used by Johnson and Swanson (1942) in tabulating data from mixograms made on the National Swanson-Working recording dough mixer or mixograph. A simple plan was also given by Shellenberger (1940).

The wide variations in curves obtained from flours of different wheats were shown by Swanson (1939). He also gave a short résumé of the development of recording dough mixers as well as a discussion of the meaning of some curve characteristics. A more complete treatment of the development of dough mixers together with diagrams for the interpretation of curves has been made by Bailey (1940).

In order to be practical a diagram must be simple to construct yet comprehensive enough to furnish a basis for description and interpretation. Such diagrams are presented in Figure 1. The upper two curves or mixograms are from Tenmarq flours and the lower two are from Chiefkan flours. The two mixograms on the left are from high-protein and the two on the right are from low-protein flours. The five main characteristics of a mixogram are: (1) the degree of the incline or developing slope, (2) the time to reach the maximum height,

[1] Contribution No. 83 Department of Milling Industry.

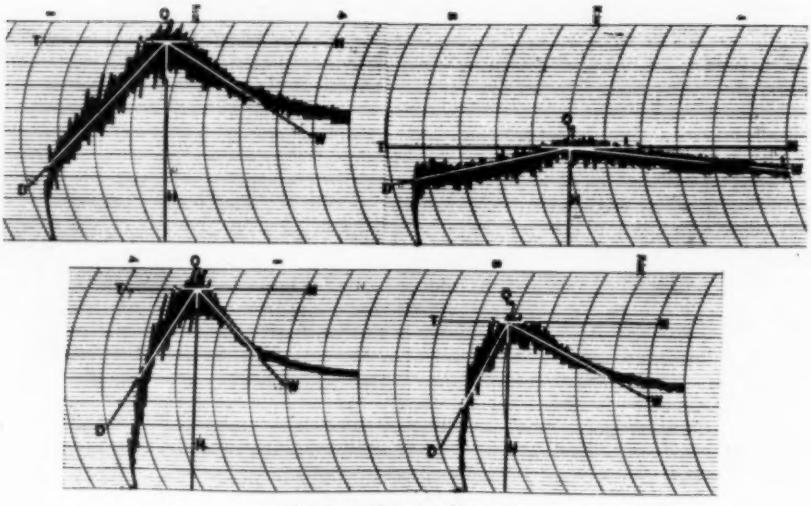

Fig. 1. Mixogram diagrams.

(3) the magnitude of this height, (4) the degree of the decline or weakening slope, and (5) the angle made by the developing and weakening slopes. Others may be used, such as the general width of the mixogram, especially at the top and last part of the weakening slope. Moreover, the area under the mixogram to optimum consistency may be used to indicate energy input, but for the present these characteristics are not included.

The line *DO* is drawn in the center of the developing slope and the line *OW* in the center of the first part of the weakening slope. From the intersection of these two lines, *O*, is dropped the perpendicular line *H*. Also through the point *O* is drawn the horizontal line *TON*, extending toward the left to the arc on which the mixogram originates and toward the right a convenient distance.

The line *DO* is more simple to draw on mixograms like the two upper than on mixograms like the two lower. More consistent measurements will be obtained if the lines *DO* and *WO* are begun in the center of the upper parts of the developing and weakening slopes.

The line *TO* measures the approximate time in minutes and tenths required to develop a dough to maximum consistency or minimum mobility. The mixograms in the diagram were made with a mixing rate of 98 rpm, and the chart paper moved at such a rate that approximately one minute was required for the pen to pass from one arc to another. If smooth or a differently ruled chart paper is used, the horizontal space over which the pen moves in one minute must be established. The line *HO* gives the height in units of the chart. This is divided by horizontal lines into ten major units, each of which is

divided into five. Thus any distance from the bottom of the chart to the point *O* may be read in parts of 100.

The height of the mixogram is determined primarily by the protein content and secondarily by the water absorption of the flour. The amount of absorption influences the stiffness of the dough and hence the degree of torque given the bowl by the movements of the pins through the dough. This torque registers the resistance of the dough to the process of mixing and is mostly influenced by the protein or gluten content. The process of mixing presumably develops the protein into gluten strands or fibrils and arranges these in a three-dimensioned, continuous network in the dough. The quantity of protein determines the density of this network, and the quality determines the strength of the gluten fibrils. Quality is, however, more readily revealed in the other mixogram characteristics and hence is only a minor factor in mixogram heights.

The angle *DOT* indicates rate of dough development and hence is called "development angle." This, as well as the other angles in this diagram, is measured in degrees. The magnitude of angle *DOT* will be negatively correlated with the mixing time. If the complimentary angle *DOH* is used, the correlation will be positive.

The rate of development as shown by the angle *DOT* is not necessarily the same as the time of development indicated by the line *TO* since the time required to reach the top of the mixogram is determined not only by the slope of the line *DO* but also by the height *H*. Thus mixograms may have little or no variation in the degrees of angle *DOT*, but vary greatly in the magnitude of lines *TO* and *OH*.

Resistance to the movement of the upper pins starts almost immediately as is shown by the rapid swing of the pen away from the lower edge of the chart. As soon as the flour particles make contact with water, wet gluten develops. The to-and-fro movement of the recording pen starts as soon as the gluten forms. The rate of wetting differs in flours as can be seen by comparing the upper two mixograms in Figure 1 with the two lower. The to-and-fro swing of the pen is not due to the elastic properties, but to the plastic, since such a movement may be obtained from a mixture of starch and water as has been shown by Markley (1937).

The range of mixing tolerance is measured by the angle *DOW* formed between the developing line *DO* and the weakening line *WO*. The slower the rate of development and the rate of breakdown of the dough, the larger will be this angle. Large angles, *DOW*, like the two upper curves of the diagram, indicate much wider range in tolerance to mixing than the smaller angles of the two lower curves.

The rate of dough weakening is measured by the angle *WON* formed by the slope of the weakening line *WO* and the horizontal line *ON*. The last half of the mixograms of many of the flours are concave and others are more nearly straight. The first part of the weakening slope is the most significant and hence the line *WO* is drawn in its center. The rate at which the dough breaks, indicated by the slope of the line *WO*, is related to the variety and the protein content. In low-protein flours the angle *WON* will be smaller than in high-protein flours of the same variety. But with the same protein content the angle *WON* will vary in different varieties.

Since the sum of the three angles is 180 degrees, it follows that their magnitudes are interrelated. Particularly is the magnitude of *DOW* inverse to the magnitudes of *DOT* and *WON*.

There are several other characteristics which could be included in the mixogram diagram, but these are seemingly of secondary importance. The general width of the mixogram depends so much on the spring tension and viscosity of the oil in the shock absorber and its adjustments that it has little meaning beyond what can be seen by inspection and comparisons of mixogram types. The width of the mixogram at the point *O* tends to increase with larger amounts of protein.

The rate and amount of narrowing of the down slope has considerable significance since these are mostly variety characteristics. In some varieties this narrowing is much greater than in others and in most cases it is inverse to the protein content. As a rule the narrowing is negatively correlated with the weakening angle *WON*.

Literature Cited

Bailey, C. H.
1940 Physical tests of flour quality. Wheat Studies by the Food Research Institute **16**: 243–300.

Johnson, J. A., and Swanson, C. O.
1942 The testing of wheat qualities by recording dough mixer curves obtained from sifted wheat meals. Cereal Chem. **19**: 216–229.

Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. Cereal Chem. **16**: 733–752.

Markley, M. C.
1937 The colloidal behavior of flour doughs. I. The thixotropic nature of starch-water systems. Cereal Chem. **14**: 434–436.

Shellenberger, J. A., and Libby, J. J.
1940 A physical dough-testing method applied to flour mill stream analysis. Northwestern Miller, November.

Swanson, C. O.
1937 Wheat and flour quality. Burgess Publishing Company, Minneapolis, Minn.
1939 Variation in dough-development curves. Cereal Chem. **16**: 625–643.

—— and Clark, Rowland J.
1936 Testing flours by the recording dough mixer. Northwestern Miller, November.

—— and Working, E. B.
1933 Testing the quality of flour by the recording dough mixer. Cereal Chem. **10**: 1–29.

EFFECTS OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. II. WETTING DURING HARVEST[1]

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(Read at the Annual Meeting, May 1942)

A previous investigation (Swanson, 1941b) showed that wetting wheat, either before or after threshing, lowered the test weight and decreased the vitreous condition. Since test weight and vitreous appearance (in contrast with bleached and mealy appearance) are two important factors in grain grading, exposure to rain during harvest seriously affects the grade. The decreases in test weight, however, did not produce correspondingly lower flour yields. The baking values also did not correlate with the commercial grade nor with the extent of the wetting treatment. An explanation was given of the physical changes that take place.

Samples from Millers and Farmers

Advantage was taken of the latter part of the harvest of 1941 in Kansas, which was unusually wet, to study the effects of wetting on the physical and other properties of weathered and nonweathered wheat. Samples of wheat were obtained from millers and farmers, 34 from the former and 51 from the latter. Two samples were solicited from each one with the request that one sample should represent wheat which had received little or no wetting during harvest, and that the other should be wheat that had been exposed to considerable rain with consequent bleaching and lowering of test weight. A fine response was received. Some farmers were able to send only one, but several sent more than two.

Tests Made

The samples were put through the laboratory wheat cleaner, and test weights determined. Test-weight data, together with 75-g subsamples, were sent for official grading to Mr. Martin Schuler of the Kansas City office of the Federal Grain Inspection Department. Mr. Schuler's report is included in Tables I and II.

The internal texture of the grain was studied by using a barley kernel cutter which cuts 50 kernels at a time so that the exposed sections can be observed. Counts were made of cut sections that appeared distinctly mealy or vitreous. Those which did not fall in

[1] Contribution No. 84, Department of Milling Industry.

TABLE I

MILLERS' SAMPLES—GRADING, TEST WEIGHTS, TEXTURE AND MALTOSE VALUES, FLOUR PROTEIN, TEXTURE, AND MILLING TESTS

Serial No.	Origin of samples, county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
			%	%	*lbs*	*lbs*	%	%	%	%	*mg*	%	%	%
	NOT WEATHERED													
1859	Saline	1DHW	90.0	—	62.1	63.7	2.67	97	1	2	161	12.7	71.7	0.43
1849	Kansas City, Mo.	1DHW	94.5	—	61.7	63.6	1.67	97	1	2	164	12.8	70.0	0.43
1857	Barton	1HW	64.0	—	61.6	62.9	2.67	73	9	18	156	11.0	70.0	0.46
1843	McPherson	1HW	74.0	—	61.5	63.0	2.01	59	19	22	171	10.6	70.6	0.46
1845	Sedgwick	1HW	69.0	—	61.3	63.7	1.67	66	24	10	138	10.8	71.2	0.44
1861	Ellsworth	1DHW	82.0	0.1	61.1	63.8	2.67	88	2	10	166	11.6	70.7	0.44
1863	Barton	1HW	63.0	—	61.1	63.1	3.01	79	2	19	151	11.1	71.6	0.46
1851	Russell	1HW	55.0	—	60.8	62.6	2.00	68	15	17	161	11.4	70.3	0.42
1839	Wyandotte	1DHW	93.5	—	60.6	63.0	3.02	93	4	3	152	13.3	71.5	0.40
1855	Wyandotte	1DHW	77.0	1.0	60.2	62.7	2.67	87	7	6	166	11.9	70.0	0.44
1831	Dickinson	1HW	64.0	—	60.0	62.4	1.67	74	11	15	247	11.9	71.7	0.45
1847	Saline	2HW	60.0	—	59.9	62.4	2.67	85	10	5	161	11.3	71.7	0.42
1837	Ford	2DHW	94.0	—	59.7	61.1	1.34	82	15	3	151	13.6	66.9	0.43
1841	Sedgwick	2DHW	87.0	—	59.5	62.3	2.00	90	5	5	174	11.2	70.0	0.44
1832	Dickinson	2HW	61.0	—	59.2	61.6	1.99	58	21	21	264	11.7	71.8	0.44
1853	Saline	2DHW	82.0	—	58.9	62.1	2.34	91	4	5	138	12.2	68.6	0.48
1833	Rice	2DHW	78.0	—	58.6	62.4	1.67	97	2	1	195	10.8	71.0	0.43
1835	Harvey	3DHW	96.0	—	57.8	61.0	2.67	96	2	2	201	11.6	70.0	0.45
Average		—	76.89	—	60.31	62.63	2.25	82.2	8.6	9.2	173.2	11.75	70.52	0.44

TABLE I—*Continued*

Serial No.	Origin of samples, county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
			%	%	*lbs*	*lbs*	%	%	%	%	*mg*	%	%	%
	WEATHERED													
1852	Russell	2HW	57.0	—	59.1	61.3	1.67	23	38	39	135	11.2	72.3	0.40
1844	McPherson	2HW	38.0	1.5	58.4	60.0	4.70	29	29	42	145	10.5	71.1	0.43
1864	Barton	3HW	73.0	2.0	57.7	60.4	2.68	45	35	20	154	12.8	72.2	0.43
1840	Wyandotte	3HW	46.6	—	57.1	59.6	4.00	7	8	85	158	13.0	73.0	0.39
1858	Barton	3YHW	20.0	0.2	57.1	59.9	3.01	29	12	59	145	10.3	72.2	0.43
1850	Kansas City, Mo.	3HW	33.0	—	56.5	59.1	3.33	28	22	50	140	11.7	71.7	0.40
1842	Sedgwick	3HW	44.0	3.2	56.2	57.6	5.69	27	29	44	171	11.5	69.2	0.43
1856	Wyandotte	3HW	34.0	0.2	56.1	58.8	3.33	34	32	34	140	12.0	71.3	0.44
1836	Harvey	4HW	70.0	—	55.7	58.7	2.33	10	44	46	182	11.4	72.7	0.43
1860	Saline	4HW	26.0	2.5	55.7	58.5	2.33	36	32	32	148	12.5	72.0	0.43
1848	Saline	4HW	26.0	—	55.5	58.2	3.00	28	28	44	145	12.0	72.0	0.44
1854	Saline	4HW	26.0	—	55.5	57.8	3.01	19	46	35	135	12.9	71.6	0.43
1834	Rice	4YHW	10.0	—	55.3	58.5	2.01	5	7	88	176	10.0	71.9	0.43
1862	Ellsworth	4HW	55.0	—	55.0	57.4	7.12	34	30	36	135	13.2	68.1	0.47
1838	Ford	4HW	30.0	—	54.8	57.8	2.00	18	42	40	116	14.4	71.0	0.39
1846	Sedgwick	4HW	25.5	—	54.8	57.7	3.02	20	41	39	135	11.2	67.8	0.42
Average		3HW	38.38	—	56.28	58.83	3.33	24.5	29.7	45.8	147.5	11.91	71.26	0.424

TABLE II

FARMERS' SAMPLES—GRADING, TEST WEIGHTS, TEXTURE AND MALTOSE VALUES, FLOUR PROTEIN, AND MILLING TESTS

Serial No.	Origin of samples, county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
	NONWEATHERED		%	%	*lbs*	*lbs*	%	%	%	%	*mg*	%	%	%
1872	Marion	1DHW	99.0	Trace	64.1	66.1	1.67	100	0	0	292	12.1	71.7	0.40
1905	Ellis-Red Chief	1DHW	99.5	—	64.1	66.1	2.36	100	0	0	280	13.0	71.3	0.45
1866	Ford	1DHW	99.0	—	64.0	65.9	1.48	100	0	0	254	12.8	72.5	0.42
1903	Ellis-Chiefkan	1DHW	99.6	—	63.3	65.9	1.01	100	0	0	248	12.7	75.8	0.42
1886	Ellsworth	1DHW	91.0	—	62.7	64.6	1.34	97	0	3	217	11.9	73.2	0.47
1867	Ford	1DHW	99.0	Trace	62.4	64.9	1.27	97	2	1	237	12.4	71.0	0.42
1913	Barton	1DHW	93.2	—	62.3	65.0	1.70	100	0	0	207	11.6	73.5	0.47
1894	Scott	1DHW	99.0	Trace	61.1	63.1	4.35	98	2	0	154	15.6	71.2	0.51
1902	Ellis-Early B. H.	1HW	60.0	—	61.0	63.6	1.68	82	14	4	116	13.6	72.8	0.44
1881	Pratt	1DHW	75.0	—	60.8	63.3	1.34	87	4	9	171	10.9	72.8	0.45
1896	Pawnee	1HW	46.0	—	60.8	63.6	2.03	55	8	37	188	9.9	73.3	0.47
1868	McPherson	1DHW	75.0	—	60.3	62.7	3.02	74	14	12	192	11.0	69.8	0.45
1887	Ellsworth	1DHW	86.0	—	60.1	61.9	2.02	82	11	7	204	12.4	75.1	0.46
1906	Ellis-Tenmarq	1DHW	98.0	—	60.0	62.9	3.38	99	1	0	182	12.9	73.0	0.40
1899	Kingman	2HW	64.0	0.5	59.7	62.6	1.83	78	5	17	183	10.1	70.8	0.49
1901	Ellis-Blackhull	2DHW	88.0	—	59.6	63.2	2.02	95	2	3	171	13.1	74.1	0.45
1904	Ellis-Nebred	2DHW	91.0	—	59.5	62.8	2.03	98	2	0	191	14.3	74.6	0.41
1907	Ellis-Turkey	2DHW	96.0	—	59.5	62.9	1.35	100	0	0	195	13.2	71.6	0.41
1909	Cheyenne	2HW	89.0	0.8	59.4	61.6	1.71	65	29	6	218	13.9	73.7	0.53
1911	Cheyenne	2HW	74.0	—	59.2	62.4	2.70	82	5	13	188	13.9	76.4	0.48
1891	Reno	2DHW	80.0	0.4	59.1	63.1	4.36	86	3	11	176	10.7	71.1	0.47
1879	Decatur	2DHW	85.0	—	58.5	61.2	2.00	92	6	2	251	14.5	72.0	0.48
1880	Mitchell	2DHW	77.0	—	58.2	61.5	2.00	70	19	11	135	14.2	71.3	0.49
1877	Thomas	3DHW	78.0	—	57.3	60.3	3.02	96	2	2	154	13.5	73.8	0.51
1870	Stafford	3DHW	91.0	—	56.7	69.9	2.33	29	63	8	168	11.9	74.0	0.43
1889	Decatur	3DHW	81.0	—	56.5	59.4	3.36	88	10	2	185	12.9	71.5	0.48
Average		1DHW	85.13	.07	60.39	63.48	2.21	86.5	7.8	5.7	198.3	12.65	72.7	0.456

TABLE II—*Continued*

Average		1DHW	85.13	.07	60.39	63.48	2.21	86.5	7.8	5.7	198.3	12.65	72.7	0.456

TABLE II—*Continued*

Serial No.	Origin of samples, county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
			%	%	*lbs*	*lbs*	%	%	%	%	*mg*	%	%	%
	WEATHERED													
1869	McPherson	1DHW	45.13	—	60.0	62.4	3.22	29	9	52	166	9.5	72.3	0.45
1873	Marion	2HW	51.0	—	58.9	61.7	2.68	61	13	26	188	9.8	70.5	0.42
1895	Scott	2DHW	90.0	3.5	58.7	60.7	1.67	63	27	10	151	16.7	71.2	0.49
1897	Pawnee	2HW	28.0	—	58.7	61.2	3.39	19	13	68	179	10.0	73.6	0.49
1882	Pratt	2HW	57.0	—	58.5	61.5	3.03	57	13	30	166	10.1	72.4	0.44
1898	Pawnee	2HW	69.0	—	58.4	60.9	3.03	17	22	61	192	9.9	74.1	0.51
1888	Ellsworth	2DHW	79.0	—	58.2	60.9	2.03	62	30	8	182	13.0	74.7	0.44
1908	Ellis	4HW	32.0	—	57.7	60.8	1.35	45	36	19	198	11.2	70.7	0.40
1910	Cheyenne	3HW	60.0	—	57.6	60.3	5.08	23	60	17	195	14.5	71.9	0.52
1912	Cheyenne	3HW	58.0	—	56.7	60.1	3.05	23	25	52	156	13.0	77.6	0.47
1892	Reno	3YHW	9.0	—	56.2	58.5	3.38	1	5	94	201	9.5	74.7	0.44
1914	Mitchell	3DHW	75.0	—	56.2	59.7	3.07	59	11	30	201	11.5	72.7	0.51
1880a	Mitchell	3DHW	—	—	56.1	—	2.99	51	38	11	144	14.5	73.1	0.48
1865	Morris	4HW	67.0	0.2	55.8	59.2	2.68	36	42	22	166	11.9	73.8	0.42
1893	Reno	4YHW	7.0	—	55.8	58.6	4.07	2	9	89	198	9.6	73.9	0.43
1890	Decatur	4HW	33.0	—	55.7	58.7	3.36	11	42	47	185	12.0	73.5	0.47
1900	Kingman	4HW	30.0	—	55.7	58.6	1.94	10	22	68	145	10.4	75.2	0.49
1871	Stafford	4HW	32.0	—	55.3	58.2	3.69	18	28	54	248	10.7	72.8	0.48
1915	Mitchell	4HW	36.0	—	55.3	59.1	3.04	54	15	31	195	11.8	72.6	0.51
1878	Thomas	4HW	41.0	5.3	54.5	58.7	4.00	60	25	15	347	14.2	71.0	0.48
1885	Wabaunsee	4DHW	77.0	—	54.5	58.3	3.02	33	41	25	158	11.7	71.1	0.51
1884	Pratt	4HW	46.0	—	54.4	58.0	3.02	37	44	19	158	10.0	68.8	0.45
Average		3HW	48.86	.43	56.8	59.81	3.06	34.3	25.8	39.9	187.2	11.61	72.81	0.47
	SAMPLES OF SOFT OR RED WHEAT													
(1) 1874	Douglas	1RW	—	—	62.7	65.1	1.80	30	51	19	104	9.6	65.0	0.36
(2) 1875	"	1RW	—	—	61.1	62.1	1.01	0	0	100	93	9.3	68.4	0.32
(2) 1876	"	SGRW	—	68.7	57.5	60.2	1.34	2	2	96	88	9.1	68.8	0.47
(2) 1883	"	3RW	—	—	56.1	59.3	2.02	15	57	28	166	11.7	74.1	0.43
Average		—	—	—	59.3	61.7	1.54	12	27	61	113	9.9	69.1	0.40

either of these two classifications were counted as semivitreous. By making counts in duplicate and adding, the numbers were expressed as percentages.

Fifteen-hundred-gram portions of each sample were scoured in the experimental scourer and then milled on the Buhler mill [2] to obtain percentages of flour. Flour ash was determined in order to check on the milling operation. Test weights taken before and after scouring gave data on losses from weathered bran coats. The scouring losses are only partially indicative, since these also include broken kernels.

The flour samples were also analyzed for moisture, protein, and diastatic activity. The absorptions used in making the curves on the recording dough mixer were calculated from the protein and the moisture percentages. On the basis of dough-mixer-curve characteristics and other data, 28 samples of flour were selected for baking tests. In selecting these samples it was planned to have in each pair one unweathered and the other weathered.

Grain Grades, Test Weights, Texture Percentage, Protein Percentage, Milling Data, and Diastatic Activity

The milling and analytical data on the samples received from the millers are given in Table I. Similar data on samples obtained from farmers are shown in Table II. The origin of the samples is given by counties (or Kansas City), and for a few of the farmers' samples the variety is also given. The test weights of the cleaned wheats were used in grading. The data in Tables I and II were arranged in the order of decreasing test weights, with those for nonweathered samples in the first group and those for the weathered in the second group of each table. The classifications "nonweathered" and "weathered" were made according to the information given by the donors of the samples.

On the basis of test weights the two groups merge into each other. The average of all the test weights of the nonweathered samples from the millers was 4 pounds higher than for the weathered samples. For the farmers' samples, this difference was 3.6 pounds. Most samples had no damage and only a few had over 1%. While more samples among the weathered wheats had some damage, there were also a few among the nonweathered that had slight amounts of damage.

The percentage of vitreousness as determined in grain grading varied greatly within the different groups. A few samples among the nonweathered group had as low vitreous percentages as some of the samples among the weathered group. The average of the vitreous

[2] Credit is due Mr. Don E. Fleming, student assistant, for making the milling tests.

percentages of the nonweathered samples from millers was twice as great as that of the weathered samples and for the farmers' samples almost twice as great.

Since the figures for numerical grades are so largely dependent on test weights it follows that the grades of the nonweathered and the weathered samples should overlap as did the test weights. By far the larger portion of the nonweathered samples fell into the No. 1 grade, however, and the larger portion of the weathered samples fell into the No. 3 and No. 4 grades.

Effects of Scouring

The test weights taken on the scoured samples were in all instances greater than those obtained on the cleaned samples. The increase in test weights due to scouring was not essentially different for the nonweathered and the weathered samples. This increase in test weight was due partly to the removal of loose branny matter and removal of light chaffy material, thus facilitating closer packing. The scouring losses were considerably greater for the weathered samples, probably a result of more breaking of softened kernels as well as a looser bran.

Effects of Weathering on Internal Texture

The texture counts were based on the operator's judgment. It is not difficult, however, to pick out the kernels whose sections are distinctly vitreous, and those which show distinctly mealy, interiors. The difficulty comes with those which fall between these two classifications. Hence, those samples that have a comparatively large semivitreous count may tend either toward the more vitreous or toward the more mealy. It has not seemed practical to make more than these three divisions in the internal texture counts.

The average percentage of vitreous kernels in the millers' samples was 3.3 times as large for the nonweathered as for the weathered, and the mealy percentage 5 times as great for the weathered. In the farmers' samples the average vitreous percentage was 2.5 times as great in the nonweathered as in the weathered, and the mealy percentage was 7 times as great in the weathered. Several of the farmers' nonweathered samples were 100% vitreous.

As was indicated previously (Swanson, 1941b) the disappearance of the vitreous texture and the development of the mealy texture is caused by the swelling induced by wetting. Swelling disturbs the internal compact condition and after the water has escaped, vacuoles are left which influence the manner of light reflection. These vacuoles decrease the specific gravity and hence the test weight.

Effects of Weathering on Percentage of Flour

The percentages of flour were computed on the basis of the weight of the cleaned wheat sample. The addition of tempering water slightly increased the amounts of flour obtained, but this addition was nearly the same for all the samples. The percentages of flour as computed on the results obtained in milling cannot have the same accuracy as is accorded chemical determinations. Fifteen grams of difference in amount of flour obtained from 1500 grams of wheat will change the flour 1%. The hang-up between samples may easily vary this much. However, trends and averages are significant, especially when considered in connection with the figures for ash, which serve as a check on the milling separations.

The average percentage of flour obtained from the millers' weathered samples was somewhat larger, with a lower ash, than from the nonweathered samples. The average flour yield of farmers' samples was somewhat larger than that of the millers', and there was no difference between weathered and the nonweathered. The decrease in test weight, with consequent lowering in grade due to wetting during harvest, did not have a corresponding effect upon the flour yield.

Relationships of Grades to Internal Texture and Milling Results

The data from Tables I and II were grouped under the various wheat grades, and the averages of the four grades, together with maxima and minima for each grade, are given in Table III. The maxima and the minima vary within very wide limits in the four numerical grades. As seen in Tables I and II each numerical grade includes hard red winter (HRW) and hard winter (HW). The soft red winter (SRW) were not included in the averages. The test weights of the grades ranged within the following limits: No. 1, 4.8 lbs; No. 2, 1.6 lbs; No. 3, 1.8 lbs; and No. 4, 1.4 lbs. The differences between the maximum test weight in No. 2 and the minimum in No. 1 was 0.1 lb, which was also the difference between the maximum in No. 3 and the minimum in No. 2. In No. 4 the maximum test weight was 0.3 lb below the minimum in No. 3. Because of this larger variation within than between grades, it is easily possible that one group within a grade was little different from another group in the next lower or higher grade.

The average scouring losses were markedly greater in grades 3 and 4 than in 1 and 2. Both the maxima and the minima increased from No. 1 grade down to No. 4. The average percentages of mealy kernels increased as the grades became lower, as a result of weathering effects.

TABLE III

RELATIONSHIP OF GRADES TO TEXTURE AND MILLING RESULTS, AVERAGES WITH MAXIMA AND MINIMA

Description	Grading			Test weights		Scouring loss	Internal texture			Milling	
	Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy	Flour	Ash
		%	%	lbs	lbs	%	%	%	%	%	%
Average	1HRW	74.80	0.04	61.53	63.70	2.18	79.0	7.9	13.1	71.5	0.44
Maximum		99.6	1.0	64.8	66.1	4.35	100.0	51.0	100.0	75.8	0.42
Minimum		—	—	60.0	61.9	1.01	—	—	—	65.0	0.36
Average	2HRW	72.82	0.29	59.01	61.80	2.36	69.6	13.6	16.8	72.0	0.46
Maximum		96.0	3.5	59.9	63.2	4.70	100.0	38.0	68.0	76.4	0.48
Minimum		28.0	—	58.3	60.0	1.34	17.0	—	—	66.9	0.43
Average	3HRW	51.91	0.35	56.84	59.67	3.21	40.3	25.6	34.1	72.5	0.45
Maximum		96.0	3.2	57.9	61.0	5.69	96.0	63.0	94.0	77.6	0.47
Minimum		—	—	56.1	57.6	1.35	1.0	2.0	2.0	70.0	0.45
Average	4HRW	37.50	0.47	55.24	58.35	3.16	25.4	31.6	43.0	71.8	0.45
Maximum		70.0	5.3	55.8	59.2	7.12	60.0	46.0	89.0	75.2	0.49
Minimum		7.0	—	54.4	57.4	1.94	2.0	7.0	15.0	67.8	0.42

There were no essential differences in percentages of flour obtained from the different grades. The yields varied widely within each grade and were even wider in grades 1 and 2 than in 3 and 4. That the milling operation itself is a factor in percentage of flour yield is shown by the ash figure corresponding to the maxima and minima for flour yield. Although the ash figures were lower for the minimum flour yields than for the maximum, the differences in ash were not correspondingly as great as in the yield percentages. Hence these flour percentages were primarily dependent on the flour-yielding capacity and only secondarily on the milling operation as far as that is reflected in the ash figures.

An analysis of the data presented in Tables I, II, and III indicates that the wheat grade was no indication of potential flour yield of these samples.

Effect of Weathering on Mixogram Characteristics

The moisture and protein percentages of the flours served as a basis for the calculation of water absorptions used for making the mixograms (Swanson and Johnson, 1942) and also for the baking of selected samples. The absorptions were determined by means of a graph based on experience with a large number of flour samples. This work had established the fact that absorption within a class of flours is a function of protein content on the 15% moisture basis (Johnson, 1942).

Great variations were observed in the mixogram patterns, indicating that numerous varieties were represented among these wheat samples. With only a few exceptions each pair of mixograms, representing nonweathered and weathered wheat, was from the same variety as far as could be determined from the patterns. Accordingly the measurements on these mixograms were grouped on the same basis as the data from Tables I and II. The characteristics of the mixograms were measured according to the plan presented by Swanson and Johnson (1943).

While the mixogram patterns, as indicated by these lines and angles, depend mostly on characteristics inherent in the variety, yet within the same variety they will be influenced by weathering. After the data on the measurement of mixogram characteristics had been assembled in the same groupings as in Tables I and II and also by grades, great variations were noticed within each group and grade. It therefore seemed best to present only the averages as given in Table IV. The figures for the averages of moisture, protein, and absorption are included since these are related factors.

The effects of weathering on mixogram characteristics were more evident in the samples obtained from farmers than in the samples obtained from millers. The general effect of weathering was to increase the time of development, decrease the height of the mixogram, increase the angles of development and tolerance, but decrease the angle of weakening. Similar changes in mixogram characteristics due

TABLE IV

MIXOGRAM CHARACTERISTICS OF SAMPLES USED IN STUDY

	Moisture	Protein flour	Absorption	Mixogram measurements (Swanson and Johnson, 1943)				
				Time of development TO	Height OH	Development angle DOT	Tolerance angle DOW	Weakening angle WON
	%	%	%	*min*	*units*	*deg*	*deg*	*deg*
Millers' samples								
Nonweathered	14.1	11.8	62.2	3.0	59	37	120	23
Weathered	13.7	11.9	62.6	3.0	58	35	124	21
Farmers' samples								
Nonweathered	13.7	12.7	63.3	2.6	62	47	106	27
Weathered	13.3	11.6	62.8	3.8	54	26	137	17
Averages from the wheat grades								
Grade 1	13.7	11.8	62.4	2.7	60	42	113	25
Grade 2	13.6	12.2	62.9	3.1	59	36	122	22
Grade 3	13.5	12.0	62.7	3.1	58	34	126	21
Grade 4	13.5	11.8	62.7	3.5	54	29	134	17

to moisture were noticed in previous investigations (Swanson, 1941*a*, 1941*b*) although at that time these more precise mixogram measurements were not used. The average figures based on the grades show the same general tendency as has just been noted. This would follow from the fact that most of the nonweathered samples fell in grades 1 and 2 and the weathered in grades 3 and 4.

Response to Further Wetting

The data presented and the discussions indicate that the weathered samples had had various degrees of wetting. What further changes would take place as a result of additional wetting? To determine this, 100-g portions of all the samples, both weathered and unweathered, were placed in beakers, covered with water and allowed to soak at room temperature for three hours. The water was then drained off and the wheat placed on layers of paper toweling held in shallow pans. After complete air drying, the test weights were taken by the micro method (Swanson, 1942). For better comparison the test weights of the original wheats were also taken by this method. The internal texture was studied, using the barley cutter.

The averages of the results obtained as based on various groupings are given in Table V. The unweathered samples decreased much

TABLE V

CHANGES IN TEST WEIGHT AND INTERNAL TEXTURE DUE TO SOAKING THE SAMPLES

Grouping	Test weight			Vitreous		Mealy	
	Before	After	Loss	Before	After	Before	After
	lbs	*lbs*	*lbs*	%	%	%	%
Nonweathered, millers'	60.7	54.8	5.9	84	16	9	56
Weathered, millers'	56.5	52.9	3.6	27	11	44	61
Nonweathered, farmers'	60.6	55.9	4.7	83	18	7	37
Weathered, farmers'	57.4	53.9	3.5	36	8	36	54
Test weights 60–65.1	61.8	56.2	5.6	82	20	9	46
" " 58–59.9	59.0	54.7	4.3	65	9	19	52
" " 56–57.9	56.7	53.3	3.4	34	10	39	52
" " 54–55.9	55.2	51.8	3.4	29	9	40	55
Vitreous 75–100%	60.9	55.8	5.1	92	20	4	37
" 50– 74	59.2	54.6	4.6	63	10	18	53
" 25– 49	56.9	52.9	4.0	32	10	34	58
" 0– 24	56.5	53.2	3.3	14	6	55	66

more in test weight than the weathered. The higher test weights as well as the more vitreous group of wheats also decreased more in test weight than the lower test weight and more than the mealy samples.

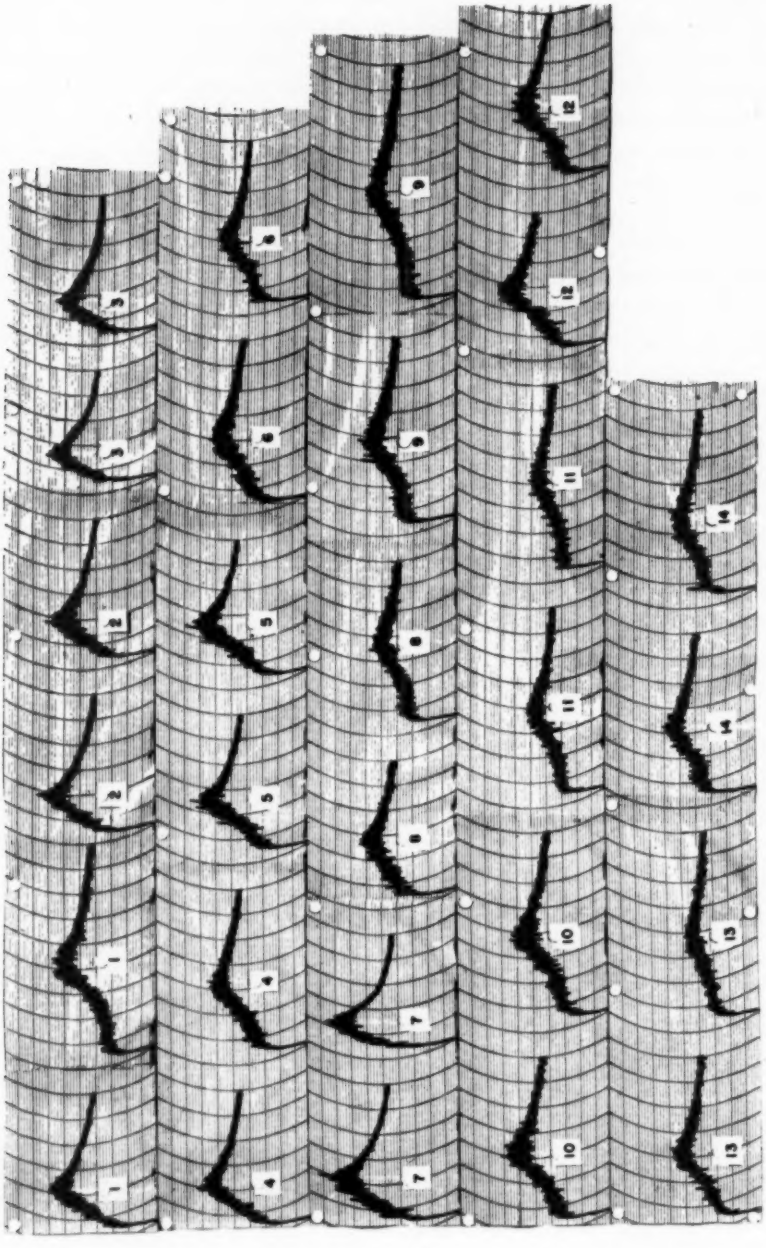

Fig. 1. Mixograms from samples of flour used in baking.

In internal texture there was a much greater change in the nonweathered than in the weathered. The higher test weight showed a greater decrease in the vitreous percentage and a greater increase in the mealy than the less vitreous wheats. On wheats which have once been subjected to wetting, additional wetting will have much less effect than on wheats which have not been wetted. How much change will take place on successive wettings will be shown in a future paper.

Mixogram Characteristics of Samples Used for Baking

Fourteen pairs of samples were selected for baking. The pairs were so chosen that one sample in each pair represented wheat from the same source that was not weathered and the other a sample that was weathered. The mixograms from all the flours served as a basis for selecting those pairs in which the main patterns of both mixograms in a pair were similar. Mixograms of these pairs are shown in Figure 1. In each pair the mixogram from the nonweathered wheat precedes that of the weathered and the order of the pairs is that of the nonweathered samples in Table VII, which gives the baking data.

The measurements of the mixograms shown in Figure 1 are given in Table VI. Those from nonweathered wheats are in the upper group and those from weathered in the lower. The averages show greater differences between unweathered and weathered than are shown by the averages in Table IV. This would be expected since those pairs of samples were selected for baking which had the larger differences between the weathered and nonweathered.

Results of Baking Tests

Results of baking tests of weathered and nonweathered samples are presented in Table VII.[3] Test weights, protein percentages, and internal texture, vitreous and mealy, are included for convenient comparisons. Since there was a considerable variation in percentage of protein, which is known to be the most important factor influencing loaf volume, the loaf volumes were reduced to a uniform protein basis. These calculated loaf volumes are found in the next to the last column of Table VII and are the basis for arrangement of the data in the table. The baking formula used was:

Flour 100 g	Salt 1.5 g
Water according to absorption	Sugar 6 g
Yeast 2 g	Bromate 3 mg
Milk solids 4 g	

Previous experiments had shown that a rich formula is better for indicating effects from moisture.

[3] Credit is due Mr. John A. Johnson for performing the baking tests.

TABLE VI

MIXOGRAM CHARACTERISTICS OF SAMPLES USED FOR BAKING

No. on Fig. 1	Time of development	Height	Development angle	Tolerance angle	Weakening angle
	min	*units*	*deg*	*deg*	*deg*
			NONWEATHERED		
1	2.2	62	55	99	26
2	1.9	65	54	92	34
3	1.6	63	54	89	32
4	2.3	65	50	102	28
5	2.6	65	50	87	43
6	3.3	58	33	128	19
7	2.0	76	53	88	39
8	2.9	58	34	115	31
9	3.8	56	24	143	13
10	3.2	61	34	128	18
11	4.0	47	19	147	14
12	2.8	64	44	112	24
13	3.2	53	33	125	22
14	3.2	52	27	136	17
Average	2.8	60.4	40.6	113.6	25.7
			WEATHERED		
1	4.3	60	28	132	20
2	1.9	61	52	100	28
3	1.7	59	57	94	29
4	3.3	58	31	133	16
5	2.6	67	49	96	35
6	3.2	54	28	136	16
7	1.2	77	66	62	52
8	3.8	50	21	143	16
9	4.9	56	20	148	12
10	3.9	56	30	133	17
11	4.4	46	17	152	11
12	3.3	55	31	132	17
13	3.9	45	18	151	11
14	3.7	50	22	145	13
Average	3.3	56.7	33.6	125.5	20.9

The baking data on texture of grain and crumb color were essentially the same for nonweathered and weathered samples and hence are not included in Table VII. Figure 2 shows the relationship of the loaf volumes, on the basis of uniform proteins, from nonweathered and weathered samples. Loaf volumes averaged larger for the weathered and were larger in each pair but two; in one of these the difference was within the experimental error. For pair No. 7, sample No. 1894

nonweathered and No. 1895 weathered, the loaf volumes were 1,010 cc and 923 cc, respectively, although the protein of the former was lower than that of the latter. The test weights were 61.1 and 58.7. The weathering had therefore mostly affected the test weight. While there were differences in the vitreous and mealy percentages, they were not large. A closer examination of the mixograms (Fig. 1) of

TABLE VII

SUMMARY OF DATA FROM SAMPLES USED IN BAKING ARRANGED BY INCREASING LV/PROT IN NONWEATHERED SAMPLES

Sample No.	No. on Fig. 1	Test weight	Vitreous	Semi vitr	Mealy	Protein	Mixing time	Loaf vol	$\frac{LV \times 10}{prot}$	Diastatic activity
		lbs	%	%	%	%	*min*	*cc*	*cc*	*mg*
UNWEATHERED										
1886	1	62.7	97	0	3	11.85	2.2	660	557	217
1877	2	57.3	96	2	2	13.50	1.9	805	596	154
1911	3	59.2	82	5	13	13.85	1.6	830	599	188
1839	4	60.6	93	4	3	13.25	2.3	830	626	152
1837	5	59.7	82	15	3	13.55	2.6	875	646	151
1889	6	56.5	88	10	2	12.85	3.3	830	646	185
1894	7	61.1	98	2	0	15.60	2.0	1010	647	154
1835	8	57.8	96	2	2	11.55	2.9	755	654	201
1870	9	56.7	29	63	8	11.90	3.8	788	662	168
1843	10	61.5	59	19	22	10.60	3.2	705	665	171
1899	11	59.7	78	5	17	10.05	4.0	695	692	183
1845	12	61.3	66	24	10	10.80	2.8	772	715	138
1833	13	58.6	97	2	1	10.75	3.2	778	724	195
1857	14	61.6	73	9	18	10.95	3.2	807	737	156
Average		59.6	81	11.5	7.4	12.2	2.8	796	655	172.4
WEATHERED										
1888	1	58.2	62	30	8	12.95	4.3	880	680	182
1878	2	54.5	60	25	15	14.20	1.9	910	641	347
1912	3	56.7	23	25	52	13.00	1.7	818	629	156
1840	4	57.1	7	8	85	13.00	3.3	863	664	158
1838	5	54.8	18	42	40	14.35	2.6	1025	714	116
1890	6	55.7	11	42	47	12.00	3.2	815	679	185
1895	7	58.7	63	27	10	16.65	1.2	923	554	151
1836	8	55.7	10	44	46	11.40	3.8	770	675	182
1871	9	55.3	18	28	54	10.65	4.9	723	679	248
1844	10	58.4	29	29	42	10.50	3.9	755	719	145
1900	11	55.7	10	22	68	10.35	4.4	730	705	145
1846	12	54.8	20	41	39	11.15	3.3	828	743	135
1834	13	55.3	5	7	88	9.95	3.9	733	737	176
1858	14	57.1	29	12	59	10.25	3.7	740	722	145
Average		56.3	26.1	27.3	46.6	12.2	3.3	822	682	176.5

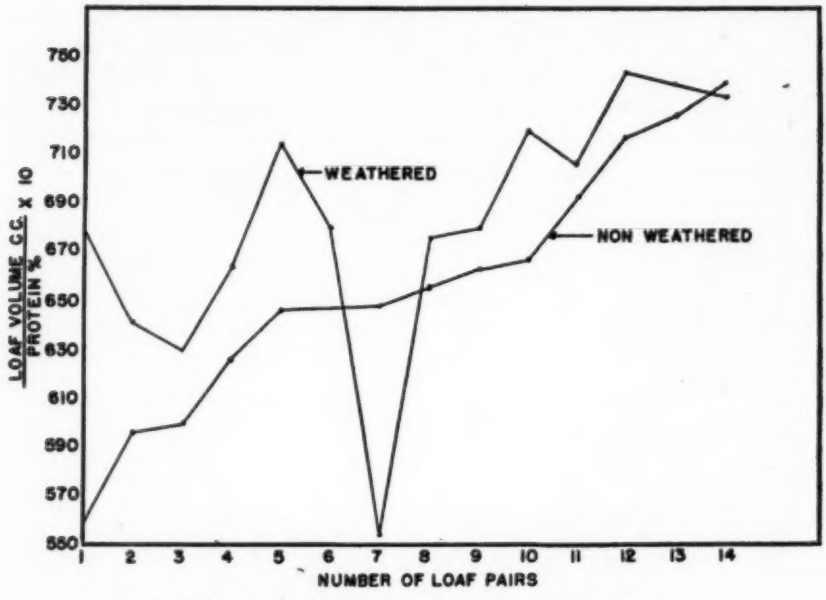

Fig. 2. Loaf volumes from weathered and nonweathered samples.

this pair indicated that the unweathered sample was of the Turkey or Blackhull type and the weathered was characteristically Chiefkan. This explains why this pair is out of line with the others.

Maltose Values in Relation to Weathering and Vitreousness

Diastatic activities were determined on all the flour samples but the results did not seem to bear any direct relationship to the weathering. The maltose values of the flours used in baking are found in the last column of Table VII. The average diastatic activity of the

TABLE VIII

DIASTATIC ACTIVITY IN RELATION TO WEATHERING AND VITREOUSNESS

Sample group	Number of samples	Diastatic activity		
		Maximum	Minimum	Average
		mg	*mg*	*mg*
Nonweathered, millers'	17	247	138	168
Weathered, millers'	17	264	116	155
Nonweathered, farmers'	26	292	104	193
Weathered, farmers'	21	347	88	182
Vitreous 100%	6	292	195	246
" 75–100%	32	292	116	189
" 50– 74%	18	347	135	191
" 25– 49%	15	171	104	154
" 0– 24%	16	248	88	163

weathered samples was somewhat higher than that of the nonweathered, but not enough to account for the higher loaf volumes.

The averages as well as the maximum and minimum figures for all the maltose values of the nonweathered and weathered as well as of four groups based on the percentages of internal vitreousness are given in Table VIII. These data indicate clearly that the weathering process as a whole did not increase the maltose values. The data also indicate that the maltose values in the highly vitreous kernels averaged higher than in those less vitreous. Among the farmers' samples were six 100% vitreous. The maltose values of these ranged from 195 to 292 and the average of the six was 246, which is considerably higher than any of the averages in Table VIII.

Discussion

Previous investigations (Swanson and Fenton, 1932; Swanson, 1934, 1941a, 1941b) have indicated that the effect of water alone, if heating and mold growth do not occur, may increase the loaf volumes. If heating occurs, and as a rule this is accompanied by mold growth, the gluten structure is injured and the result is a lowering of loaf volumes. It was also shown (Swanson, 1935) that the maltose value is not increased unless a moisture content of about 30% or more persists over a considerable period. The increase in maltose value is apparently an accompaniment of the germination process. In only a few instances did the evidence from the maltose values indicate that these weathered samples had undergone the process of germination. That this process greatly increases the maltose value was shown in the investigation just cited. A wet harvest when combines are used produces results that are different from those with wheat exposed experimentally in shocks to wet weather. As long as the wheat is supported by the standing stems, the moisture from rain does not last long enough to start the process of germination which is likely to occur in the wetted shocks.

Loaf volumes depend on the balance of the forces produced in the expanding gas bubbles, and on the resistance in the gluten mesh during dough fermentation and baking. If this resistance is too great, the gas is unable to effect the desired expansion with resulting decreased volume and coarse texture. If the resistance is too small too much expansion takes place which also results in a too open texture.

The treatment of the wheat grain with moisture, such as takes place in a wet harvest, has a mellowing effect on the gluten. The resistance is decreased and hence a larger loaf volume results from the same gas pressure. This mellowing apparently results from a slight weakening of the gluten strands, and the process is analogous

to what takes place in the natural aging which is known to improve the baking qualities of new wheat. Moisture and increased temperature hasten this process. In weathering which occurs before the wheat is harvested, temperature is a negligible factor. Heating of wheat in a bin is dangerous since it may very easily go too far.

These weathered wheats were then analogous to samples which were much older biologically than the nonweathered samples, but not too old to give good baking performance.

Summary

The latter part of the 1941 harvest season was unusually wet in Kansas. This afforded an opportunity to study the effects of wetting during harvest on grain grades, test weights, internal textures, and milling and baking values. Nonweathered and weathered wheat samples were obtained from millers and farmers.

The most apparent effects of weathering were lowering of test weight, decreasing the external vitreous appearance, depressing the grade, decreasing the percentage of internal vitreousness, and increasing the percentage of the internal mealiness.

The percentages of flour averaged as high from the weathered wheats as from the nonweathered, without a corresponding change in ash. There were no essential differences in the percentages of flour obtained from the different wheat grades.

The mixograms indicated that weathering lengthens the time of dough development, slightly depresses the height of the curve, decreases development angle and increases the tolerance angle, but decreases the angle of weakening.

The loaf volumes, adjusted in protein, were larger for the weathered than the nonweathered with only one notable exception. In this instance the curve pattern indicated that the weathered wheat was Chiefkan and the nonweathered was Turkey or Blackhull. The larger loaf volumes of the weathered samples were not due to the higher maltose values, since differences between averages of nonweathered and weathered were not significant. The average maltose value of the flours from six 100% vitreous wheats was about one-third higher than the average of the weathered samples.

The larger loaf volumes obtained from the weathered samples as compared with the nonweathered samples were due to changes similar to those which take place in aging, and which give an improvement over new wheat in baking qualities.

The data presented indicate that weathered wheats which show no other damage are unduly penalized by the prevailing grain grading standards.

Literature Cited

Johnson, John A.
1942 Master's thesis, K.S.C.
Swanson, C. O.
1934 Some factors involved in damage to wheat quality. Cereal Chem. **11**: 173–199.
1935 Some factors that affect the diastatic activity in wheat. Cereal Chem. **12**: 89–107.
1939 Moisure and air space as factors in test weight. Milling Production, N. W. Miller, Vol. **4**, pp. 2 and 30.
1941a Effect of low temperature in preventing damage to wheat stored with high moisture. Cereal Chem. **18**: 299–315.
1941b Effect of moisture on the physical and other properties of wheat. Cereal Chem. **18**: 705–729.
1942 A micro method for determining test weight. Cereal Chem. **19**: 468–470.
—— and Fenton, F. C.
1932 The quality of wheat as affected by farm storage. Tech. Bul. No. **33**, Kans. Agr. Exp. Station.
—— and Johnson, John A.
1943 Description of mixograms. Cereal Chem. (preceding paper, this issue).

FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. V. GLUTEN PROTEIN AS THE MAIN FACTOR AFFECTING THE PATTERN OF MIXOGRAMS[1]

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Recording dough mixer curves or "mixograms" (Swanson and Johnson, 1943), made with and without the presence of surface-active or wetting agents, were presented in a previous paper (Swanson and Andrews, 1942). The principal effects of the surface-active agents were to lengthen the time of development of the dough, decrease the angles of development and weakening slopes, and decrease the rate of breakdown of the dough. The view was expressed that these effects were results of the action of the surface-active agents on the protein and that their action may have been some form of protein denaturation. Reducing the interfacial tension would aid water penetration into the large polyhedral protein molecules. The protein deformation would be aided by the severe action of the pull-fold-repull type of mixing, causing the protein particles to change more to the laminar and the fibrillar forms, and thus facilitate the increase of the extent of surface of the three-dimensioned fibrillar gluten network in the dough.

Water constitutes about 40% to 45% of the dough mass (Swanson, 1937, p. 180). About one-half to two-thirds of this water is in the free condition (Skovholt and Bailey, 1935; Vail and Bailey, 1940). The presence of the surface-active agents in the water, probably in both the

[1] Contribution No. 89, Department of Milling Industry, and No. 275, Department of Chemistry.

free and the bound forms, could, because of their effect on adhesional tensions, contribute to the alteration of the mixogram characteristics.

Probably the greatest effect of surface-active agents is on the protein of gluten, as has been stated. That the percentage of protein in the flour is one of the principal factors influencing mixogram heights was shown by Swanson (1941) and also by Larmour, Working, and Ofelt (1939). The protein content and its inherent varietal quality had the greatest influence on the main mixogram patterns. The percentage of protein in a dough made from average hard winter wheat flour is about 7% of the dough mass (Swanson, 1937, p. 180), yet its influence on mixogram patterns is apparently greater than that of the starch and also of the water when present in such amounts as to give the dough its proper consistency. Since starch constitutes about 40% of the mass of dough from average hard winter wheat flour, its influence should not be disregarded.

The purpose of the investigation presented in this paper was to determine to what extent the main varietal patterns of mixograms are altered or persist when the gluten protein is weakened by admixture with starch, ethyl alcohol, or pyridine in the presence and absence of the surface-active agent Aerosol OT (Swanson and Andrews, 1942). The general plan was to determine the changes in the pattern of mixograms (1) by mixing varying amounts of wheat starch with high-protein Tenmarq and Chiefkan flours, (2) by successive additions of alcohol, and (3) by using mixtures of starch and finely ground dried gluten so proportioned as to produce the same protein content as the high-protein flours. The effect of the action of Aerosol OT was determined by its addition to all these combinations. Pyridine was used since it has no effect on starch but has a pronounced weakening effect on the gluten protein.

The flours used were a 15.6% protein Tenmarq, a 15.6% Chiefkan, a series of Tenmarq flours decreasing in protein from 15.3% to 7.9%, and a series of Chiefkan decreasing in protein contents from 14.6% to 8.7%. The dried glutens and the corresponding starches were obtained from the two high-protein Tenmarq and Chiefkan flours. The wheat starch was furnished by the Huron Milling Co., Huron, Michigan.

Mixogram Patterns as Influenced by Admixture of Increasing Amounts of Starch and Alcohol

The effects on mixogram patterns of lowering the protein content by successive additions of wheat starch and also of weakening the protein by adding alcohol are shown in Figure 1. The flours were the high-protein Tenmarq and Chiefkan. The proportions of flour and starch,

percentage of absorption, milliters of 95% alcohol and water used are given in the legend for Figure 1.

The most apparent effect of the addition of starch was the successive lowering of the heights of the mixograms. The time of development or minutes to reach the top was not changed within each variety, but between varieties the time of development for Tenmarq was significantly over twice as long as for Chiefkan, indicating that the time of development was affected almost wholly by quality and practically not at all by quantity of the gluten protein. The effect of alcohol was similar to that of starch in lowering the heights of the mixograms, except that the tops were made more round by the larger amounts of alcohol and the time of development was noticeably shortened for Tenmarq but not for Chiefkan. There was also a greater smoothness from the use of the 5 and 7 ml of alcohol. The disintegrating action of alcohol made the liquid properties associated with viscosity more prominent than the solid properties associated with plasticity.

The effect of the starch is due to decreasing the density or quantity of gluten strands in the three-dimensioned gluten mesh in the dough, while the effect of alcohol is from a weakening of the mesh. Alcohol has a dispersing or solvent effect on gliadin, one of the main constituents of gluten.

When the proportion of flour became 5 parts to 30 of starch (last mixograms of lines 1 and 3, Fig. 1) the plastic and viscous properties of the starch were so prominent as to conceal all influence of the gluten protein. The time of development was only that required to intermingle the water with the starch and the small amount of flour. When the proportion of flour was 10 parts to 25 of starch (mixograms in lines 1 and 3, No. 6), the influence of the gluten was evident. In the ratio of 10 to 25, the calculated protein content was 4.46% and hence near the lower limit at which dough characteristics are shown in a mixogram from a mixture of high-protein flour and wheat starch.

In a previous paper (Swanson and Andrews, 1941, Table 3 and Fig. 4) it was shown that the developing and weakening slopes almost disappeared in the mixograms from the lowest-protein flours, or those in the range of 8% or 9%. This effect indicates that the gluten protein in a low-protein flour behaves differently from the gluten protein from a high-protein flour in a mixture of flour and starch in dough development. Markley (1938) states that "It would appear that at least 7% of protein must be present if there is to be a continuous gluten structure throughout the dough at mobility levels normal to bread doughs." He implies that at this protein level in the flour there is a formation of a more or less complete film or envelope of protein over the surface of the starch granule. The principle of dough handling by the farino-

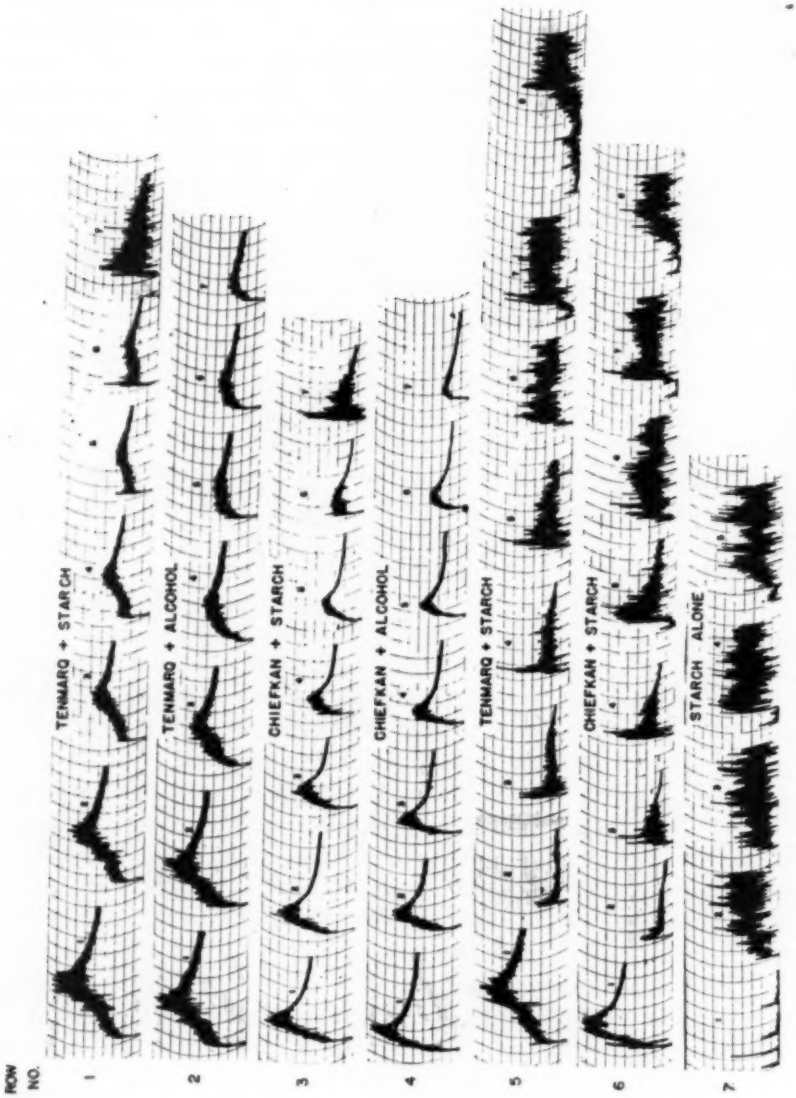

Fig. 1. *(Explanation of figure on opposite page.)*

graph, used by Markley, is different from the pull-fold-repull type of mixing in the National Swanson-Working mixograph.

The differences between the mixogram patterns of Tenmarq and Chiefkan, which persist after dilution of the gluten protein with starch or after weakening with alcohol, indicate that these patterns are determined by differences in the characteristics inherent in the gluten proteins of these varieties.

Influence of Absorption on Mixtures Containing High Starch Ratios

The amount of water absorption in mixtures of 5 parts flour and 30 parts starch has a marked influence on the characteristics of the mixograms (lines 5 and 6, Fig. 1). The mixograms numbered 2 in lines 5 and 6 were made with the same absorption as was used for the mixograms numbered 1, which were made from the flour alone. Mixograms numbered 3, 4, and 5 were made with 2% successively decreasing absorptions. When the absorption was 70% (the same as for the flour alone, mixograms in lines 5 and 6) comparatively little resistance was offered to the moving pins after water was incorporated in the mixture. The first upswing was due to the resistance of the dry areas in the mixtures. As the amount of absorption was decreased the viscous and plastic properties became more apparent, as shown by the wider swing of the pen. That there may have been some influence from the small amount of protein, 2.23%, is shown in the narrowing of the last part

Fig. 1. Effects of wheat starch and alcohol on mixogram patterns. The grams of flour are indicated by the first figures of the ratios, the grams of starch by the second figures.

TENMARQ + STARCH

Row No.								
1. Curve No.	1	2	3	4	5	6	7
Flour-starch ratios	35–0;	30–5;	25–10;	20–15;	15–20;	10–25;	5–30
Absorption %	70	69	68	67	66	65	63

TENMARQ + ALCOHOL

2. Curve No.	1	2	3	4	5	6	7
Alcohol ml	0	1	2	3	4	5	7
Water ml	24.5	23.5	22.5	21.5	20.5	19.5	17.5

CHIEFKAN + STARCH

3. Curve No.	1	2	3	4	5	6	7
Flour-starch ratios	35–0;	30–5;	25–10;	20–15;	15–20;	10–25;	5–30
Absorption %	70	69	68	67	66	65	64

CHIEFKAN + ALCOHOL

4. Curve No.	1	2	3	4	5	6	7
Alcohol ml	0	1	2	3	4	5	7
Water ml	23.8	22.8	21.8	20.8	19.8	18.8	16.8

TENMARQ, LARGE STARCH RATIOS, DECREASING ABSORPTION

5. Curve No.	1	2	3	4	5	6	7	8
Flour-starch ratios	35–0;	5–30;	5–30;	5–30;	5–30;	3–32;	2–33;	1–34
Absorption %	70	70	68	66	64	64	64	64

CHIEFKAN, LARGE STARCH RATIOS, DECREASING ABSORPTION

6. Curve No.	1	2	3	4	5	6	7	8
Flour-starch ratios	35–0;	5–30;	5–30;	5–30;	5–30;	3–32;	2–33;	1–34
Absorption %	70	70	68	66	64	64	64	64

STARCH ALONE, 35 GRAMS

7. Curve No.	1	2	3	4	5
Absorption %	66	68	70	70	70

(mixograms 4 and 5 of lines 5 and 6). This is also shown in the last mixograms of lines 1 and 3. The influence of 3, 2, or 1 grams of flour in the mixture was not apparent in the mixograms (Nos. 6, 7, and 8, lines 5 and 6).

Mixograms from Starch Alone

Mixograms in line 7 (Fig. 1) were made from starch and water. With 66% absorption (mixogram 1) the mixture was too dry and would cling to the bowl. At several intervals the machine was stopped and the mixture worked away from the side. The result was a few upswings of the pen and then the mixture again clung to the side of the bowl. That this behavior continued for a much longer time than is normally used in making mixograms was learned in several trials. The plastic properties appeared when the absorption was increased, as shown in mixograms 2 to 5, line 7. These, however, disappeared when the absorptions became too large.

It was found difficult to determine the absorptions which would produce the characteristics of mixogram 1, line 7, and of mixograms 2 to 5, line 7. The exposure of the starch to the atmosphere was a factor, since mixograms with the same absorptions varied on different days. Thus mixogram 8 in lines 5 and 6 had 64%, while 66% for mixogram 1 in line 7 was too dry. Mixtures which appeared very dry at the start, such as produced the last mixograms in lines 5 and 6, gradually developed plastic properties. The depths or thicknesses of the water films on the starch granules, which produce patterns similar to No. 1, line 7, and Nos. 2, 3, and 4, line 7, differ in an extremely narrow range.

The wide mixograms, obtained with little or no gluten proteins present, show that the width is not related to elastic properties of the dough but depends on the plastic properties. This has also been shown by Markley (1937). The magnitude of the width is determined also by the spring tensions and the viscosity of the oil in the shock absorber of the mixograph.

Effect of Aerosol OT on the Mixograms Made from Mixtures of High-Protein Flours and Starch

The mixograms in Figure 2 show the effect of Aerosol OT, with the same flour and starch ratios as were used for those in lines 1 and 3 of Figure 1. The first line of mixograms under each variety (Fig. 2) were made with flour, starch, and water. Those in the second line under each variety show the effects of 40 mg of Aerosol for each one and those in the third line, the effect of 50 mg.

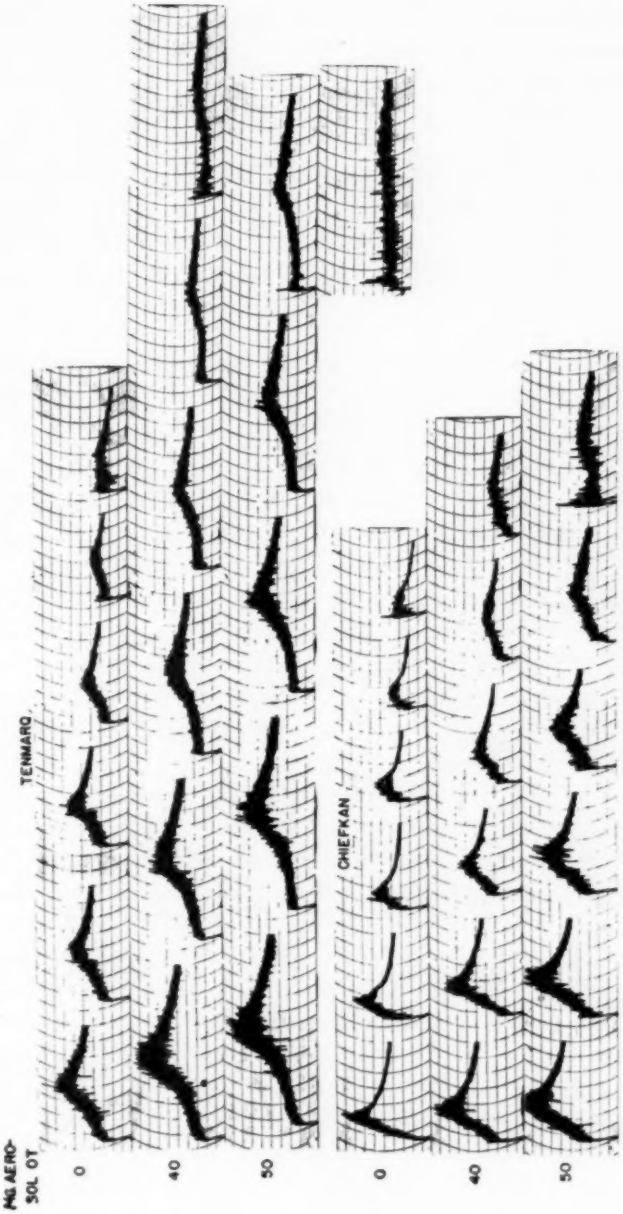

Fig. 2. Effects of Aerosol OT on flour and starch mixtures.

RATIOS OF FLOUR AND STARCH
35–0; 30–5; 25–10; 20–15; 15–20; 10–25

ABSORPTION PERCENTAGES
70, 69, 68, 67, 66, 65, for each of the six rows of curves.

The effects of surface-active agent were longer time of development, less steep developing and weakening slopes, and a tendency to lessen the rate of breakdown in the dough. The effects of the surface-active agents on the flour-and-starch mixtures were similar but not identical to the effects on flours of decreasing protein content (Swanson and Andrews, 1941).

That the surface-active agent acts mainly on the gluten protein and not on the starch is indicated by the persistence of the differences in patterns between Chiefkan and Tenmarq mixograms, not only after dilution with starch but also under the influence of the surface-active agent.

Effects of Aerosol OT on Flours of Decreasing Protein Contents and on Mixtures of High-Protein Flour and Starch of Corresponding Protein Contents

To investigate further whether the influence of the surface-active agent was mainly on the gluten protein, the mixograms in Figures 3 and 4 were obtained. Those in Figure 3 were made on a series of Tenmarq and Chiefkan flours of decreasing protein percentages. The protein percentages as well as the water absorptions for both varieties are given in the legend for Figure 3. The mixograms in the first row under each variety were made with flour and water and the second row for each variety was made with flour, water, and 50 mg of Aerosol OT. The absorptions were adjusted so that the top of the one with the highest protein content would reach near to the top of the chart. For each of the lower proteins the absorptions were reduced in order to give doughs of desired consistency as shown by the mixograms. These mixograms are similar to some shown in the previous investigation (Swanson and Andrews, 1942). It is evident that, although the surface-active agent changed the pattern of the mixograms of both Tenmarq and Chiefkan, the differences between the varieties persist.

The mixograms in Figure 4 were made from mixtures of starch with the 15.6%-protein Tenmarq and Chiefkan flours so proportioned that a series for each variety would be obtained which would have protein contents corresponding to the flours used for those in Figure 3. The mixograms in the top rows in Figure 4 under each variety were made with flours and the additions of 0, 20, 40, and 60 mg of Aerosol OT, respectively. Those in the rows below the top row were made with the flour-and-starch mixtures just mentioned, and the additions of Aerosol OT in the same amounts as for the top rows. The calculated protein contents of the flours and the mixtures as well as the absorptions used are given in the legend for Figure 4.

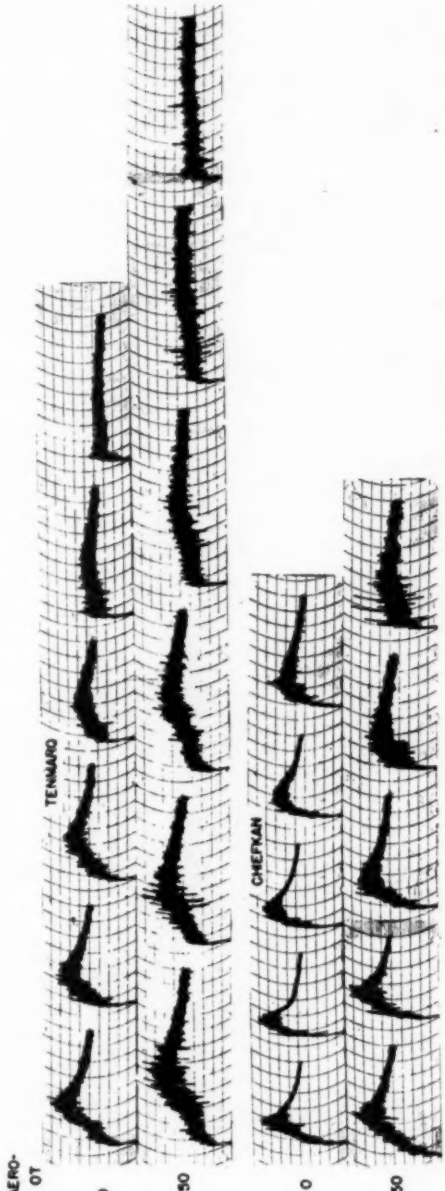

Fig. 3. Effects of Aerosol OT on flours of decreasing protein percentages.

TENMARQ						
Protein percentages	15.3;	13.3;	11.8;	10.8;	9.3;	7.9
Absorption %	65	62	59.8	58.3	56.8	56.0
CHIEFKAN						
Protein percentages		14.6;	13.1;	10.8;	9.3;	8.7
Absorption %		65	62.8	59.3	57.8	57.5

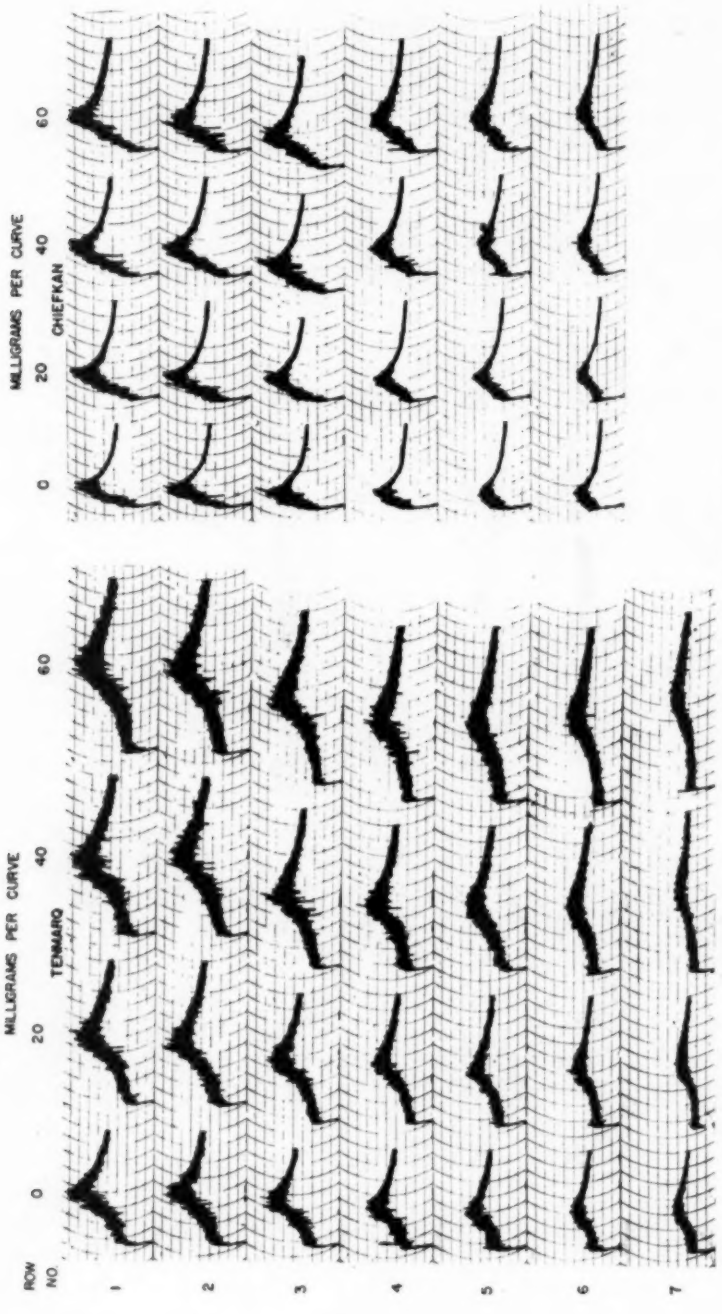

Fig. 4. (*Explanation of figure on opposite page.*)

The absorptions of the flour-and-starch mixtures were higher than of the flours of corresponding protein contents used for the mixograms in Figure 3. This was apparently due to the fact that the two flours used in making the flour-and-starch mixtures were higher in absorption than was the absorption of the highest-protein flours, which were employed for the mixograms in Figure 3. The absorption of the starch itself in these mixtures was also a factor.

The mixograms in Figures 3 and 4 show that when a high-protein flour was mixed with starch so as to decrease the protein content, the characteristics of the mixograms were not the same as those obtained from flours of the same protein content as the mixture. Mixograms of the lowest-protein flour, Tenmarq, are almost horizontal (Fig. 3). When the protein was reduced to the same level by mixing with starch (line 7, Fig. 4) the developing and weakening slopes remained distinct. There was also a greater tendency toward narrowing in the last half of those from the flour and starch mixtures, especially those of Tenmarq. In the mixograms from the flours (Fig. 3) the time to reach the top gradually increased as the protein content decreased. In the flour-starch mixtures (Fig. 4) no such increase in time of development is noted. This shows a fundamental difference in the behavior in mixing of protein from these high- and low-protein flours. The low-protein flours require more time than the high-protein flours for physical development. It is apparently not the small quantity of protein in the low-protein flours that is responsible for a longer time to reach the top. The difference is in the quality of the proteins. This also indicates that low-protein flours synthesized by mixing starch with high-protein hard wheat flour will not have the same handling qualities as the naturally low-protein flours.

Progressively longer times were required to reach the top with increasing amounts of Aerosol OT (Fig. 4), but this time did not become shorter as the protein content of the flour-starch mixtures was decreased. There was a greater tendency for the mixograms of mixtures of flour and starch (Fig. 4) to become narrow in the last half than for those made from the flours shown in Figure 3. The time to reach the top was more than twice as long for Tenmarq as for Chiefkan for

Fig. 4. Effects of Aerosol OT on flour and on flour and starch mixtures adjusted so as to have the same protein contents as the flour used for the curves in Fig. 3.

Row No.	TENMARQ		CHIEFKAN	
	Protein in flour or mixtures	Absorption	Protein in flour or mixtures	Absorption
	%	%	%	%
1	15.6	70	15.6	70
2	15.3	70	14.6	69
3	13.3	69	13.1	69
4	11.8	68	10.8	68
5	10.8	67	9.3	68
6	9.3	67	8.7	67
7	7.9	67		

all the mixograms from the mixtures both without and with the various additions of Aerosol. Thus to a large extent the characteristics of those made from flour alone persist in the flour-and-starch mixtures, which shows that it is the gluten protein that determines the patterns.

Mixograms from Mixtures of Dry, Finely Pulverized Gluten, and Starch

The mixograms presented in the preceding discussion indicate very strongly that the characteristics inherent in the gluten protein determine the main pattern, and also that a wetting agent similar to Aerosol QT acts principally on the gluten. Additional evidence was obtained from mixograms on mixtures of dry, finely pulverized gluten and starch.

Gluten was washed from doughs made from the 15.6% protein Tenmarq and Chiefkan flours. By washing the dough in a large bowl most of the starch could be recovered in three changes of water. The assembled starch-water was passed through a Sharples supercentrifuge which obtained the starch as well as the water-dispersible protein in a pasty condition. This starch was easily dried by being placed in a shallow layer in front of a fan, after which the lumps were ground to pass a 10XX flour sieve.

The wet gluten mass was picked into small pellets about the size of peas and spread on waxed paper, such as is used in wrapping bread, and allowed to air-dry. The pellets, resembling dried glue, were ground to pass a 10XX flour sieve. It was noticed that the pellets from Tenmarq and Chiefkan glutens behaved differently when resting on the waxed paper. Those from Chiefkan flattened out more and had a smoother surface, while those from Tenmarq were humped up and had a rippled surface. Protein content of the gluten and the starch obtained from these flours are given in Table I.

TABLE I

PROTEIN IN THE GLUTEN AND STARCH FROM TENMARQ AND CHIEFKAN—AIR-DRY

	Protein
	%
Tenmarq gluten	78.20
Tenmarq starch	0.79
Chiefkan gluten	72.9
Chiefkan starch	1.79

The gluten and starch were mixed in ratios that approximated the protein content of the flour alone, 35–0, and of the flour and starch mixtures 25–10 and 15–20, used for mixograms 1, 3, and 5 in Figure 2. The approximate protein percentage of the gluten and starch mixture

corresponding to 35–0 was 15.6%, that of 25–10, 11.1%, and that of 15–20, 6.7% protein.

The check mixograms obtained from flour and water, and from the gluten and starch mixtures, without and with the addition of 40 mg of Aerosol OT for each mixogram, are given in Figure 5 and the proportions as well as the absorptions are given in the legend for this figure. The checks were from the flours. Those in the three pairs of rows on the left side were from Tenmarq flour and from Tenmarq gluten-starch mixtures. Those in the three pairs of rows on the right side were from Chiefkan flour and from Chiefkan gluten-starch mixtures. The first one in each line was made from flour, the other three from gluten mixtures. The mixograms in the first line of each pair were made without Aerosol OT and those in the second line with 40 mg of Aerosol. For those in the upper pairs, plain wheat starch was used in the mixture, and for the two middle pairs, the starch obtained from the same flours as the glutens. For the mixograms in the lower two pairs, Chiefkan starch was used with Tenmarq gluten and Tenmarq starch with the Chiefkan gluten. The details of the quantities used are indicated in the legend for Figure 5.

It is apparent that the mixograms obtained from Tenmarq gluten and wheat starch are characteristically different from those obtained from Chiefkan gluten and wheat starch. The Tenmarq gluten produced mixogram characteristics associated with Tenmarq flours, and the Chiefkan gluten produced characteristics associated with Chiefkan flours. This indicates that, in addition to the preceding evidence, the characteristics of the gluten determine the patterns of the mixograms. The additions of Aerosol OT to the gluten and starch mixtures result in effects similar to those produced by the flours.

The starch and gluten mixtures, however, do not give exactly the same characteristics as obtained from the flours. This can be seen by comparing the first mixogram in each line with the second, which had an equivalent amount of protein. When the amounts of gluten were decreased and the amounts of starch increased in the mixtures, the heights were lowered similarly to those shown in Figures 2 and 4.

There were also variations in the effects of the different starches. The pure wheat starch did not give the same pattern as was obtained from the starches obtained from the flours. The Tenmarq starch produced greater variations from the pure starch than did Chiefkan starch. The addition of Aerosol OT did not conceal the differences due to the two glutens. It should be noted that the starches from these two flours contained a considerable amount of protein, and also that the protein contents of the two glutens (Table I) indicate that they contained a considerable amount of starch. From the method

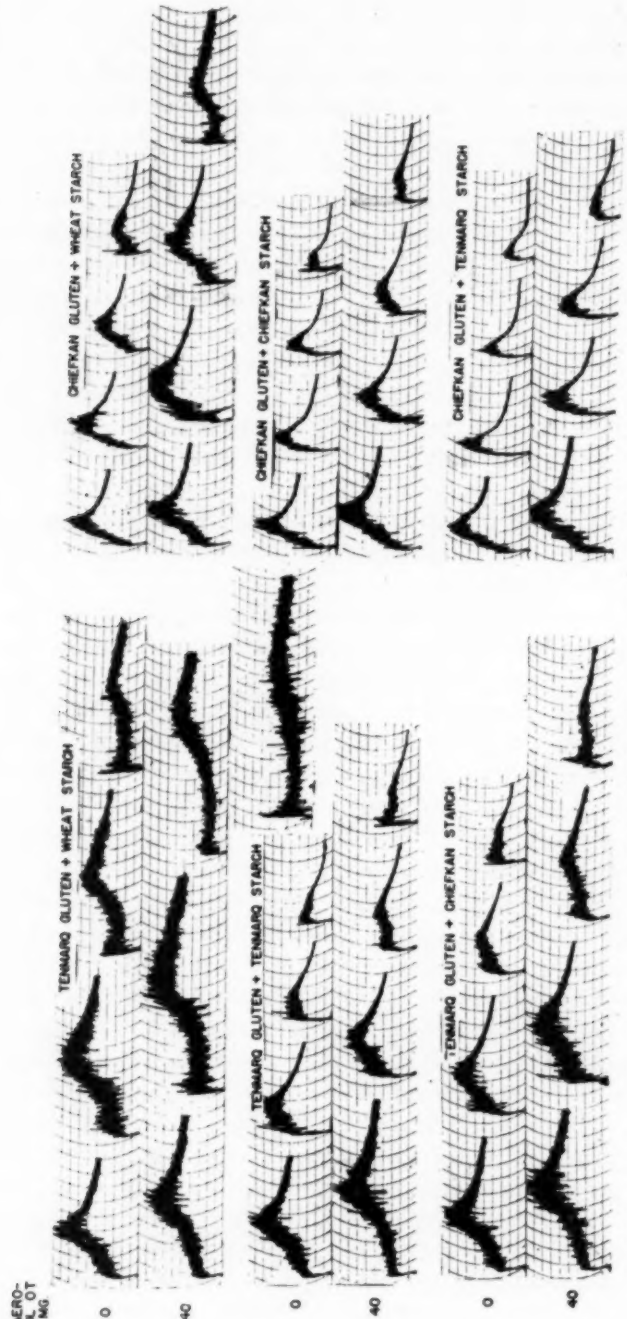

Fig. 5. *(Explanation of figure on opposite page.)*

used in the gluten and starch separations, it is inevitable that a considerable portion of the water-soluble protein would be lost in the water removed by the centrifuge. It is clear, however, from these trials, that the patterns of the mixograms from these varieties are determined mainly by the characteristics inherent in the gluten proteins.

Effect of Pyridine on Mixograms

Pyridine is an organic liquid which has no chemical effect on starch and hence it was thought worth while to ascertain what effects its presence would have on the mixogram patterns. Different amounts of a mixture of 85 ml of pyridine and 15 ml of water were added in making the mixograms. The same Tenmarq and Chiefkan flours and

Fig. 5. Comparison of mixograms from flours and from gluten and starch mixtures.

Aerosol OT mg.					
TENMARQ GLUTEN + WHEAT STARCH					
0	Grams gluten	Check	7.0	5.0	3.0
	Grams starch		28.0	30.0	32.0
	Absorption %	70	70	68	66
40	Grams gluten	Check	7.0	5.0	3.0
	Grams starch		28.0	30.0	32.0
	Absorption %	70	70	68	64
TENMARQ GLUTEN + TENMARQ STARCH					
0	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	64
40	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	66
TENMARQ GLUTEN + CHIEFKAN STARCH					
0	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	66
40	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	66
CHIEFKAN GLUTEN + WHEAT STARCH					
0	Grams gluten	Check	7.5	5.4	3.2
	Grams starch		27.5	29.6	31.8
	Absorption %	72	72	70	68
40	Grams gluten	Check	7.5	5.4	3.2
	Grams starch		27.5	29.6	31.8
	Absorption %	72	70	70	68
CHIEFKAN GLUTEN + CHIEFKAN STARCH					
0	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	72	68	66
40	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	72	68	66
CHIEFKAN GLUTEN + TENMARQ STARCH					
0	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	70	68	66
40	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	70	68	66

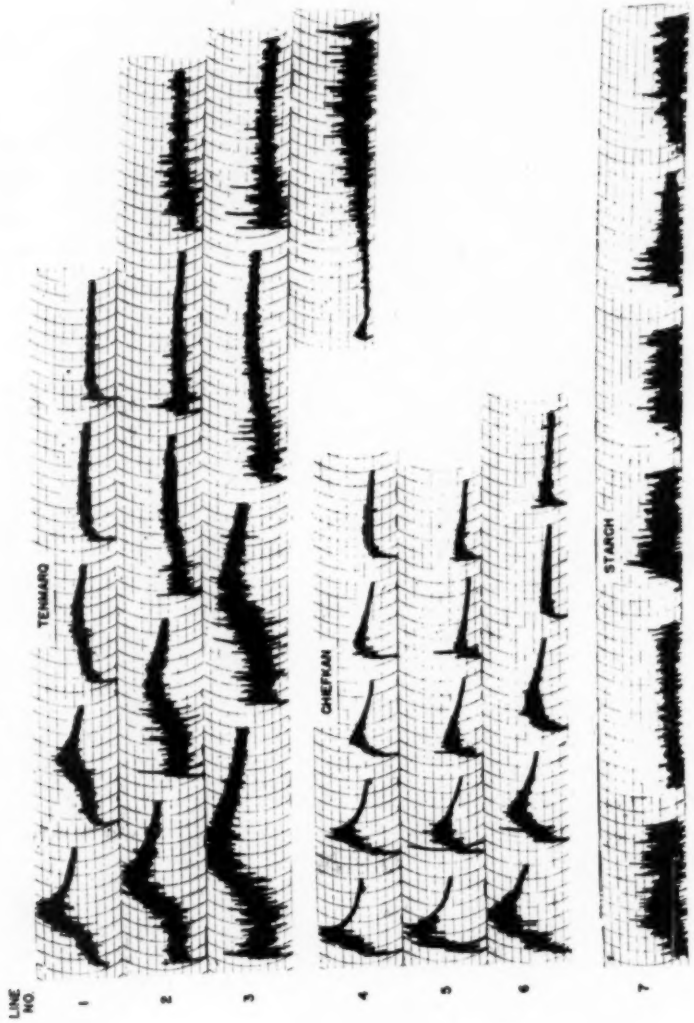

Fig. 6. (*Explanation of figure on opposite page.*)

their glutens, as well as the same wheat starch as was employed for those in Figure 5, were used.

The mixograms obtained are shown in Figure 6 and the proportions of materials are given in the legend. The upper three lines of mixograms represent Tenmarq and the next three lines Chiefkan, both 15.6% protein. The first one in each line was made without pyridine, and the remaining four curves were made with the additions of 1, 2, 3 and 5 ml of pyridine mixture, respectively. The mixograms in the first line under each variety were made with flour, those in the second line with starch and gluten used in such amounts that the mixture had the same protein content as the flour. The third line of mixograms under each variety was also made with the mixtures of starch and gluten, but also 50 mg of Aerosol for each mixogram. The absorption for all these was 70%, except for the mixture of Chiefkan gluten and starch, which was 72%.

Pyridine has a disintegrating effect on wheat gluten. The presence of 3 and 5 ml of pyridine mixture made a plastic mass instead of a dough. That this was due to its effect on the protein is indicated by the same general results on the gluten and starch mixtures as on the flours. That Aerosol OT exerts its effect in the presence of pyridine is shown by the similarities of the general patterns of the curves in lines 3 and 6 to those given in Figure 5, in which Aerosol OT was present.

Summary and Conclusions

The mixograms obtained from mixtures of high-protein flours with starch, arranged to provide a series of samples of decreasing protein contents, differed markedly from those obtained from flours of the same protein contents as the mixtures. It appears that the gluten in high-protein flours reacts somewhat differently in mixing from the gluten of low-protein flours. Mixograms made with mixtures of finely ground gluten and starch had the same general pattern as those made

Fig. 6. Effects of pyridine on mixogram patterns—pyridine mixture added for each mixogram 0, 1, 2, 3, and 5 ml, respectively, except line 7.

Line No.			
	TENMARQ		
1	Flour, pyridine—70% abs.		
2	Gluten-starch mixtures + pyridine—70% abs.		
3	Gluten-starch mixtures + pyridine + Aerosol OT—70% abs.		
	CHIEFKAN		
4	Flour, pyridine—70% abs.		
5	Gluten-starch mixtures + pyridine—72% abs.		
6	Gluten-starch mixtures + pyridine—72% abs.		
	STARCH ALONE		
7	Wheat starch − 68% abs.	Wheat starch + 3 ml pyridine − 68% abs.	Tenmarq starch − 64% abs.
	Tenmarq starch + 3 ml pyridine − 64% abs.	Ch. starch − 62% abs.	Ch. starch + 3 ml pyridine − 62% abs.

from mixtures of high-protein flours and starch. The mixograms from Chiefkan gluten and starch had patterns similar to those obtained from Chiefkan flour and similarly with Tenmarq flour.

Pyridine has a disintegrating effect on gluten. The additions of small but increasing amounts produced mixograms similar to those obtained from flours of progressively decreasing protein contents. Pyridine added to gluten and starch produced the same general pattern as was obtained from its addition to flours.

The mixograms presented in the various figures indicate very clearly that gluten-protein is the main factor in the determination of patterns of mixograms and also that the surface-active agent Aerosol OT has very little if any effect on the starch. The reduction of the interfacial tensions in the dough by the surface-active agent appears to be a factor in promoting inter-spreading among the starch granules and intra-penetration of the water into the protein micelles or gluten particles.

It can be concluded from this investigation that the starch has very little if any influence on the main mixogram patterns, and that the gluten-protein is the main factor.

Literature Cited

Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. Cereal Chem. **16**: 733–752.
Markley, M. C.
1937 The colloidal behavior of flour doughs. I. The thixotropic nature of starch water systems. Cereal Chem. **14**: 434–436.
1938 Colloidal behavior of flour doughs. III. Studies upon the properties of flour-starch-water systems. Cereal Chem. **15**: 438–444.
Skovholt, O., and Bailey, C. H.
1935 Free and bound water in bread doughs. Cereal Chem. **12**: 321–355.
Swanson, C. O.
1941 Factors which influence the physical properties of dough. III. Effect of protein content and absorption on the pattern of curves made by the recording dough mixer. Cereal Chem. **18**: 615–627.
1937 Wheat and flour quality, p. 180. Burgess Pub. Co., Minneapolis.
—— and Andrews, A. C.
1942 Factors which influence the physical properties of dough. IV. The effect of surface active agents on the characteristics of curves made by the recording dough mixer. Cereal Chem. **19**: 102–120.
—— and Johnson, J. A.
1943. Description of mixograms. Cereal Chem. **20**: 39–42.
Vail, Gladys E., and Bailey, C. H.
1940 The state of bound water in colloidal gels—free and bound water in bread doughs. Cereal Chem. **17**: 397–417.

TESTING THE BAKING STRENGTH OF GLUTEN CONCENTRATES[1]

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(Read at the Annual Meeting, May 1942)

The linear relationship between loaf volume and protein content, expressed as a coefficient of regression, has been widely used in studies of wheat quality, and should serve as a satisfactory tool for examining the baking strength of gluten concentrates. If several concentrates are to be compared, each can be used to fortify one or more base flours at different protein levels, and the resulting data can be used to determine the regression coefficients for each gluten and the significance of possible differences between them. The essence of such a method is that a statistical analysis is used to obtain an objective and quantitative interpretation of the data. The first investigation made in this laboratory, involving the use of this procedure, is described in this paper.

Materials and Methods

Twenty-four high-grade Western Canadian hard red spring wheats of the 1941 crop, ranging in protein content from 11.2% to 16.7%, on which milling and baking data were available, were used in this study. Nine wheats from the lower protein range were composited to give a flour (A) of 11.5% protein; eight wheats from the intermediate protein range were composited to give a flour (B) of 13.3% protein; and seven wheats from the higher protein range were composited to give a flour (C) of 15% protein. Gluten concentrates (protein content about 63%) were prepared from flours A, B, and C, by the method of Aitken and Geddes (1938). Each base flour was fortified with each of the three glutens at nine levels to cover a range of approximately 3%. Thus there were nine sets of samples, representing combinations of three base flours and three glutens. A single loaf was baked for each sample by the malt-phosphate-bromate procedure, absorption being determined with the aid of the farinograph. The dried glutens were weighed out and added to the flours without preliminary treatment. Protein levels of the fortified flours were calculated from data on the protein contents of the base flour and added gluten. The ten loaves for each set were baked in random order on the same half day. The order in which bakings were made for the nine sets of samples was randomized among half days.

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Results and Discussion

The correlation and regression coefficients for loaf volume and protein content for the gluten-fortified flours and for the original 24 flours were calculated in the usual manner and are recorded in Table I.

TABLE I

CORRELATION AND REGRESSION COEFFICIENTS FOR LOAF VOLUME AND PROTEIN CONTENT

Combination	Correlation coefficient	Regression coefficient
		cc loaf vol. per 1% of protein
Original 24 flours	+.98	70
Flour A plus:		
Gluten A	+.93	33
Gluten B	+.94	43
Gluten C	+.91	31
Flour B plus:		
Gluten A	+.88	30
Gluten B	+.84	37
Gluten C	+.88	27
Flour C plus:		
Gluten A	+.94	45
Gluten B	+.92	41
Gluten C	+.92	37

All correlation coefficients are well above the value required for a 1% level of significance. The regression coefficients for the fortified flours show a minimum value of 27 and a maximum value of 45, the mean being 36. This mean value is considerably lower than the regression coefficient for the original flours, 70. Application of the t test shows that this difference is significant, t having a value of 8.41 as compared with a required value of 2.63 for a 1% level of significance. It is thus apparent that the average difference in the loaf volumes of natural flours differing in protein content by 1% is about twice as great as the average increase in loaf volume obtained by increasing the protein content 1%, by adding dried gluten of the type used in this study. This difference indicates that gluten concentrates made by the Aitken-Geddes method are materially damaged during preparation.

The homogeneity of the regression coefficients for the nine sets of fortified flours was examined by means of the analysis of residual variance shown in Table II. The F value failed to attain the 5% level of significance. Thus in spite of the fact that 90 loaves were baked, the investigation did not demonstrate significant differences between the strength-imparting properties of the glutens or between the effects of different base flours on the regression coefficients. It

TABLE II

ANALYSIS OF RESIDUAL VARIANCE OF REGRESSION COEFFICIENTS FOR LOAF VOLUME ON PROTEIN CONTENT FOR GLUTEN-FORTIFIED FLOURS

Variation due to	Degrees of freedom	Mean square
Differences among the nine individual regressions	8	392.8
Deviations from individual regressions	72	355.5

F value found, 1.14; required for 5% level, 2.06.

should be noted, however, that it cannot be concluded that either the glutens or the base flours are identical in their effects.

The failure to obtain positive results stems from an attempt to obtain too much information from a given number of bakings. Thus if the 90 loaves had been used to study the fortification of a single flour with two glutens, it seems probable that any real differences in quality between the glutens would have been established. In a study of the kind attempted it is apparently advisable to use both a greater protein range and more levels of fortification. Both changes will increase the precision of the regression coefficient. It should be noted that baking duplicate loaves for each level of fortification is less advantageous than increasing the number of levels and baking single loaves. The latter procedure provides a more rigorous test since it takes full account of the total experimental error. It also yields a maximum number of degrees of freedom for purposes of examining the statistical significance of differences between regression coefficients.

Summary

It is shown that the average difference in loaf volume (70 cc) of natural flours differing in protein content by 1% is about twice as great as the average increase (36 cc) obtained by increasing protein content 1% by adding dried gluten prepared by the Aitken-Geddes method. Differences in quality were not demonstrated between glutens prepared from high, medium and low protein wheats, but further replication may show that such differences exist.

Literature Cited

Aitken, T. R., and Geddes, W. F.
1938 The effect on flour strength of increasing the protein content by addition of dried gluten. Cereal Chem. 15: 181–196.

THE PREPARATION AND DECOLORIZATION OF CEREAL EXTRACTS FOR NICOTINIC ACID DETERMINATION

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In the past few years several papers have been published on methods of chemical determination of nicotinic acid in biological material. The basis of all these methods is the production of a colored compound when nicotinic acid reacts with cyanogen bromide in the presence of certain amines. A source of difficulty, however, is the fact that part of the nicotinic acid may occur in the form of nicotinamide or its derivatives, which are biologically active. Since these compounds do not react with the color reagents in the same manner as does the free acid, they must first be hydrolyzed to the acid. The hydrolysis is carried out by heating the test material in strong acid or alkali. In the case of cereals this often gives rise to a large amount of pigments, and it is in the treatment of the solution to eliminate or to circumvent the effect of these pigments that the various methods diverge.

Kodicek (1940) has added acetone or alcohol to precipitate the pigment from the colored solution, the organic solvent being subsequently evaporated off. Melnick and Field (1940), Melnick, Oser, and Siegel (1941), and Waisman and Elvehjem (1941) adsorbed most of the pigment on Darco charcoal, the remaining color then being corrected for colorimetrically as a blank. These workers claim that under the prescribed conditions the charcoal adsorbs pigment but not nicotinic acid. Arnold, Schreffler, and Lipsius (1941), instead of removing the pigment, carried out the color reaction and used a selective solvent, ethyl acetate, to extract the color produced. Friedemann and Barborka (1941) have found zinc hydroxide precipitation to be useful in clearing hydrolysates of blood, urine, and feces. Perlzweig, Levy, and Sarett (1940) have shown that nicotinic acid can be adsorbed on and eluted from Lloyd's reagent, and have advocated this procedure together with lead hydroxide precipitation for decolorization of solutions for nicotinic acid determination. Dann and Handler (1941) applied this principle to the determination of nicotinic acid in animal tissues. These investigators further emphasized the desirability of using solutions for the color reaction as free from pigment as possible. They showed that the pigments may themselves react with the reagents to increase the color production, thus exaggerating the apparent nicotinic acid value. We have studied the method of these

workers and have found it, with modification, to be applicable to the determination of nicotinic acid in cereals. In our hands more nearly colorless solutions were obtained by this method of decolorization than by any other we have tried.

The danger of hydrolyzing cereal samples directly without preliminary extraction has been pointed out by Kodicek (1940), and by Waisman and Elvehjem (1941). Most workers have carried out the extraction with water in an autoclave or a boiling-water bath. We have found in the case of cereals, however, that gelatinization of the starch makes the resulting mixture difficult to handle. Bina, Thomas, and Brown (1941) have suggested the use of takadiastase at this point. But since this increases the color produced during hydrolysis, we have found it expedient to extract with tenth-normal sulfuric acid. For hydrolysis of the extract, Kodicek (1940) has shown 8% sulfuric acid to be sufficiently strong.

For the final color development we have used a modification of the method of Melnick and Field (1940) and Waisman and Elvehjem (1941), employing aniline as the amine. Dann and Handler (1941) claim that metol is more specific than aniline, but we have found this reagent to be rapid and satisfactory.

Experimental

To a sample of the finely divided material containing 100–500 μg of nicotinic acid in a 100-ml volumetric flask is added 75 ml of 0.1*N* H_2SO_4, and the mixture is placed in a boiling-water bath for one hour. The flask is agitated frequently during this period. At the end of the extraction period the contents of the flask are cooled and made to volume. The mixture is centrifuged and 20 ml of the supernatant pipetted into a 50-ml centrifuge tube. Enough concentrated H_2SO_4 is added to make the solution 1.6*N* (0.88 ml) and the tube is heated in a boiling-water bath for one hour. The contents of the tube are adjusted to pH 0.5–1.0 with 65% NaOH using methyl violet as an outside indicator.

Two and a half grams of Lloyd's reagent is then added and the contents of the tube are stirred for at least one minute. This is centrifuged and the supernatant discarded. The Lloyd's reagent is washed twice with 10-ml portions of 0.2*N* H_2SO_4, centrifuged, and the supernatant discarded. The tube is inverted after the final washing and allowed to drain well. Twelve milliliters of 0.5*N* NaOH is then added and the contents stirred for one minute or longer. This is followed by 8 ml of H_2O to make the volume of fluid 20 ml.

The contents of the tube are well mixed and centrifuged. The supernatant is poured into another centrifuge tube containing 0.72 g

of powdered Pb $(NO_3)_2$ and stirred well. This is centrifuged and the supernatant poured into a third clean, dry centrifuge tube. One drop of phenolphthalein is added, then solid K_3PO_4 until the solution is pink, followed by just enough 20% H_3PO_4 to bring the solution to neutrality. One drop should be sufficient. After centrifuging, the clear supernatant is used for the color reaction.

For the color reaction the method we use is essentially that of Melnick and Field (1940). In their method the final solution was one-third alcohol. To 3 ml of this solution were added the cyanogen bromide and aniline. To determine the color produced by a standard quantity of nicotinic acid they added 0.1 ml of alcohol containing 10 μg of the acid to the 3 ml of unknown. In our procedure 2 ml of solution is used, and to this is added 1 ml of alcohol, followed by the cyanogen bromide and aniline. For the determination of the color produced by a known amount of nicotinic acid, we substituted for the 1 ml of alcohol, 1 ml of an alcoholic solution of nicotinic acid, containing 10 μg. We have found 95% alcohol to be as satisfactory as absolute so far as the reaction and stability of nicotinic acid are concerned. In our experiments the Evelyn photoelectric colorimeter was used for color measurement. Application of the method of decolorization described above to a large number of cereals gave color blanks with L values (2 minus log galvanometer reading) seldom exceeding 0.0088. Nicotinic acid values for a number of cereals as determined by this method are shown in Table I.

TABLE I

COMPARISON OF NICOTINIC ACID VALUES OBTAINED BY HYDROLYSIS OF CEREAL EXTRACTS WITH 1.6N H_2SO_4 WITH THOSE OBTAINED BY DIRECT HYDROLYSIS OF THE WHOLE SAMPLE WITH 4N HCl

Sample	Hydrolysis of extract with 1.6 N H_2SO_4	Total hydrolysis with 4 N HCl
	μg per g	μg per g
1. Whole wheat A	65	63
2. Whole wheat B	59	61
3. Whole wheat bread	—	64
4. White flour A	13.7	18.0
5. White flour B	12.9	16.6
6. Yellow corn meal	13.3	20.2
7. White corn meal	10.5	11.2
8. Corn flakes	15.4	17.3
9. Rye crackers	29.2	29.2
10. Brown rice	56	60
11. Rice polish	—	468
12. Rolled oats A	24.0	25.2
13. Rolled oats B	21.2	—
14. Soy-wheat bread	22.7	—
15. Roasted soy beans	38.5	—
16. Peanut flour	361	—

Since hydrolysis of the whole sample with strong HCl has been suggested by a number of investigators we thought it of interest to compare values obtained by this method with those by the method described. These results are also shown in Table I. In most of the experiments hydrolysis of the whole sample with 4*N* HCl gave final solutions with as little color as those after hydrolysis of the extract with 1.6*N* H_2SO_4. However, in the case of two products, white flour and yellow corn meal, the solution for the final color reaction contained an appreciable amount of yellow pigment when the whole sample was hydrolyzed with 4*N* HCl, the *L* values of the color blanks ranging from 0.0362 to 0.0555. Coincidentally, these samples (4, 5, and 6) gave significantly higher nicotinic acid values by this method than by the hydrolysis of the extract with 1.6*N* H_2SO_4. This bears out the contention of Dann and Handler (1941) that a colored solution may exaggerate the nicotinic acid value. In the method of Melnick, Oser, and Siegel (1941) for determination of nicotinic acid in flour and bread, the final solution frequently has a considerable amount of color. We feel this to be an objection to the charcoal adsorption procedure.

It might be argued that the higher nicotinic acid values are nearer the truth as the result of a more drastic extraction procedure. To test this possibility we extracted white flour by the usual method, hydrolyzed the extract with 4*N* HCl and found the same colored solutions and exaggerated values as when the whole samples were hydrolyzed. We also hydrolyzed white flour and yellow corn meal directly with 1.6*N* H_2SO_4 and obtained colorless solutions, and nicotinic acid values no higher than those obtained by hydrolysis of the extract with this acid. This shows that the factor causing color and enhanced values was the use of 4*N* HCl, and not the direct hydrolysis. The possibility of routinely dispensing with preliminary extraction and using direct hydrolysis with 1.6*N* H_2SO_4 is under investigation.

Hydrolyses of white flour directly with 4*N* H_2SO_4 and with 1.6*N* HCl were also carried out. In each case the nicotinic acid values were increased in proportion to the amount of pigment present in the solution. Hydrolysis with 1.6*N* HCl gave a solution with a very slight color, while 4*N* H_2SO_4 gave somewhat more. The pigment resulting from hydrolyses with 4*N* H_2SO_4, however, was not nearly as great as that from 4*N* HCl, indicating that this effect is more pronounced with hydrochloric than with sulfuric acid.

Two typical recovery experiments are shown in Table II. While the recovery of added nicotinic acid is good, it must be borne in mind that good recovery is no guarantee of completeness of extraction. It is, however, an indication that no significant amount of nicotinic acid is lost in the purification procedure.

TABLE II

RECOVERY OF NICOTINIC ACID ADDED TO SAMPLES OF WHITE FLOUR

	Nicotinic acid found	Recovery
	μg	%
10 g white flour 1	152	—
10 g white flour 1 plus 100 μg nicotinic acid	246	94
10 g white flour 2	148	—
10 g white flour 2 plus 100 μg nicotinic acid	243	95

Summary

A method is described for preparing colorless solutions of cereal extracts for nicotinic acid determinations. This method is based on adsorption on and elution from Lloyd's reagent, followed by treatment with lead hydroxide. Nicotinic acid values for various cereals, as determined by this method, are presented.

Evidence is presented indicating that the use of 4*N* HCl for hydrolysis may give colored solutions and exaggerated nicotinic acid values for some cereals.

Literature Cited

Arnold, A., Schreffler, C. B., and Lipsius, S. T.
1941 Chemical determination of nicotinic acid. Ind. Eng. Chem. (Anal. Ed.) **13**: 62–63.

Bina, A. F., Thomas, J., and Brown, E. B.
1941 The determination of nicotinic acid in bread and other cereal products. Cereal Chem. **18**: 661–666.

Dann, W. J., and Handler, P.
1941 The quantitative estimation of nicotinic acid in animal tissues. J. Biol. Chem. **140**: 201–213.

Friedemann, T. E., and Barborka, C. J.
1941 A procedure for the decolorization of acid digestion mixtures for the determination of nicotinic acid. J. Biol. Chem. **138**: 785.

Kodicek, E.
1940 Estimation of nicotinic acid in animal tissues, blood, and certain foodstuffs. Biochem. J. **34**: 712–724.

Melnick, D., and Field, H.
1940 Determination of nicotinic acid in biological materials by means of photoelectric colorimetry. J. Biol. Chem. **134**: 1–16; **135**: 53.

Melnick, D., Oser, B. T., and Siegel, T.
1941 Chemical determination of nicotinic acid content of flour and bread. Ind. Eng. Chem. (Anal Ed.) **13**: 879–883.

Perlzweig, W. A., Levy, E. D., and Sarett, H. P.
1940 Nicotinic acid derivatives in human urine and their derivation. J. Biol. Chem. **136**: 729–745.

Waisman, H. A., and Elvehjem, C. A.
1941 Chemical estimation of nicotinic acid and vitamin B_6. Ind. Eng. Chem. (Anal. Ed.) **13**: 221–225.

STABILITY OF WHEAT GLUTEN DISPERSIONS TOWARD REDUCING AGENTS IN THE PRESENCE AND ABSENCE OF A GLUTEN PROTEINASE

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As part of a comprehensive program of wheat protein research in this laboratory, methods for obtaining stable gluten dispersions were required in order to ensure against alterations of the proteins during the extended time periods that might be involved in fractionation operations.

Numerous investigators have commented on the instability of glutens in alkaline solutions (Blish and Sandstedt, 1929, and others). According to Larmour and Sallans (1932), the most satisfactory method for separating wheat proteins was that first proposed by Blish and Sandstedt (1925), utilizing the solubility and relative stability of the gluten proteins in dilute acetic acid. However, Rose and Cook (1935) observed appreciable decreases in the viscosities of such gluten solutions with time, which they interpreted as indicating instability of the protein in the solvent. In a later article, the same authors (Cook and Rose, 1935) reported hydrolytic changes in acetic acid dispersions of gluten. They found that dispersions of greater stability could be obtained with two neutral reagents, urea and sodium salicylate, and that the latter was particularly useful for fractionation purposes.

In the present series of investigations, it was observed that the loss in viscosity of acetic acid dispersions of gluten could be prevented by heating to near boiling temperature for a few minutes. It may be concluded, therefore, that the hydrolytic changes in the unheated dispersions were due to a proteolytic enzyme which was destroyed by heat treatment. Not only did the heating of the gluten dispersion in acetic acid produce no coagulation or precipitation, as previously observed by Cook and Rose (1935), but there was also no evidence from salting-out behavior that the gluten properties had been significantly altered.

As a further objective of the research program, the effects of various reagents on the properties of gluten are being studied. The results with reducing reagents are of interest in connection with a controversial issue concerning the nature of the rapid and pronounced softening action produced when small quantities of certain reducing agents (cysteine, glutathione, sulfide, etc.) are added to bread dough. Jørgensen (1936) and Balls and Hale (1936a) independently suggested

that this action was attributable primarily to an activation of a flour proteinase by the reducing agent. On the other hand, Balls and Hale (1936b) and Ford and Maiden (1938), using different experimental approaches, suggested that the effect of the reducing agents was not explainable entirely on the basis of proteinase activation. Incontrovertible evidence for either viewpoint has been lacking.

The availability of gluten that has been freed from proteinase activity without apparent loss of its essential and distinctive properties makes it possible to obtain evidence bearing directly on this issue. It has now been found that reducing agents produce characteristic effects upon gluten *in the complete absence of any proteinase* as will be described below.

Experimental Methods

Gluten was obtained from commercially milled unbleached bakers' flour, the starch being removed by manipulation in tap water in the usual manner. Dispersions in acetic acid, sodium salicylate, and urea were made by adding the washed gluten to predetermined amounts of the solvent in the Waring Blendor, followed by separation of the remaining starch and a small fraction of insoluble protein in a Sharples supercentrifuge.

Viscosities were measured in modified Ostwald viscometers at 24.8°C. Unless otherwise indicated, storage experiments also were run at this temperature. The usefulness of the Sørenson formol titration method was extended by the step of adding, just prior to the titration, solid urea to the extent of approximately 20% of the aliquot used. This technique [1] prevents the precipitation of gluten during the addition of the alkaline titrating solution. The nonprotein nitrogen fraction was determined in duplicate after precipitation of the proteins with 10% trichloroacetic acid (final concentration). A semimicro Kjeldahl method was used for the determination of nitrogen; where conversion to protein was required, the 5.7 factor was employed. In order to subject gluten dispersions to approximately 100°C temperature, the samples, in small test tubes with slotted cork stoppers, were placed in a steam bath for the required length of time. A glass electrode was used for pH determinations.

Demonstration of the Enzymatic Nature of Gluten Decomposition in Dilute Acetic Acid Dispersion

Gluten dispersions in acetic acid solution (0.01–0.1*N*) showed a decrease in viscosity and accumulated increasing amounts of nonprotein nitrogen components on standing, as shown previously by Rose

[1] Suggested by Dr. Hans Lineweaver.

and Cook (1935; Cook and Rose, 1935). In addition, there were measurable increases in the alkali required for the formol titration, indicating the liberation of amino groups. However, when the original gluten solutions were heated for 5 to 10 minutes at 100°C, viscosity, formol titration, and nonprotein nitrogen remained constant or changed only slightly as compared to the unheated dispersion. The results of such observations for a dispersion in 0.1*N* acetic acid are shown in Figure 1.

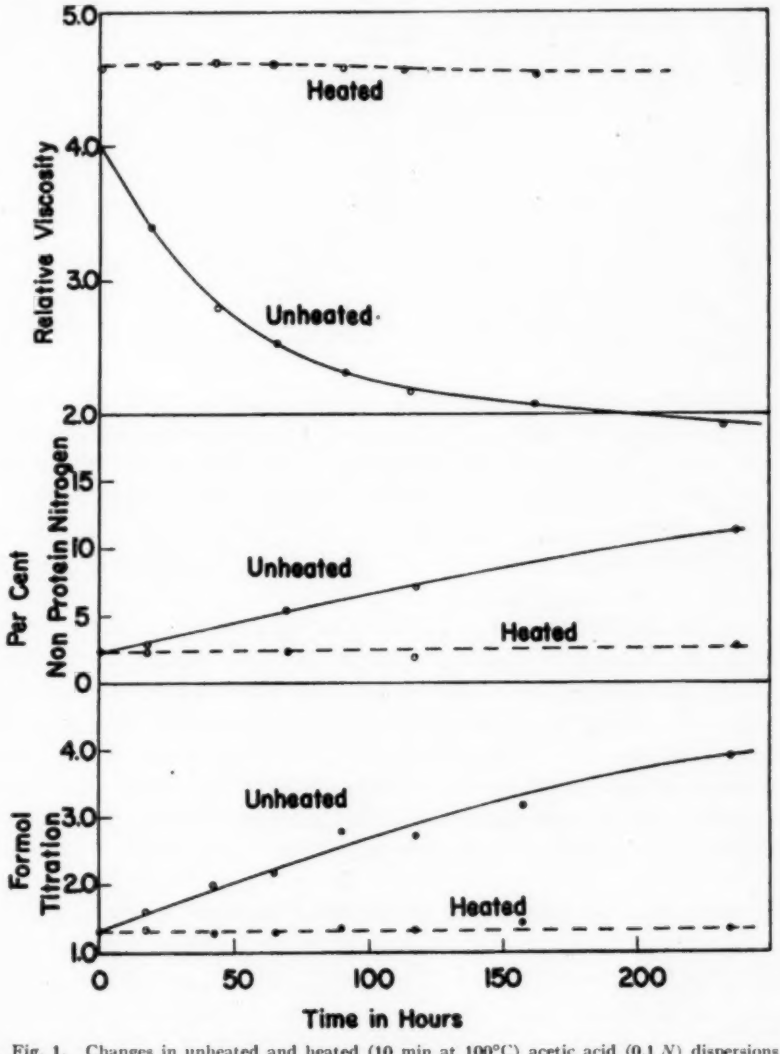

Fig. 1. Changes in unheated and heated (10 min at 100°C) acetic acid (0.1 *N*) dispersions of gluten (6.3 mg N per ml) upon standing at 25°C. Formol titration data are expressed in terms of ml of 0.1 *N* NaOH required per g of gluten.

In order to determine whether the enzyme responsible for these changes was capable of hydrolyzing heat-treated gluten dispersions, the decreases in viscosity of *mixtures* of heated and unheated gluten dispersions were followed. In all cases, the loss in viscosity was greater than that calculated on the assumption that the unheated portion only was undergoing change (Table I), indicating that the enzyme was affecting both heated and unheated proteins.

TABLE I

RELATIVE VISCOSITY CHANGES IN MIXTURES OF UNHEATED AND HEATED[1] GLUTEN DISPERSIONS[2] AT 25°C

Dispersion	1 hr	45 hrs	122 hrs	169 hrs
Unheated	4.4	3.2	2.6	2.5
Heated	4.6	—	—	4.6
Unheated + heated (1 : 1) calc.[3]	4.5	3.9	3.6	3.5
Unheated + heated (1 : 1) found	4.4	3.7	3.0	2.9
Unheated + heated (1 : 4) calc.	4.5	4.3	4.2	4.2
Unheated + heated (1 : 4) found	4.5	4.0	3.5	3.3

[1] Heated 10 minutes at 100°C.
[2] Contained 8.11 mg N per ml in 0.1*N* acetic acid; pH, 3.7.
[3] Calculated on the assumption that the relative viscosities were additive. The differences between the calculated and found viscosities are larger than the inaccuracies inherent in such an assumption.

Further analysis of such data by the method of O. Bodansky (1937), as illustrated in Figure 2, showed that for any specific change in the viscosity of mixtures of heated and unheated gluten dispersions the product of the concentration of unheated gluten and time was approximately constant. This relation holds only for enzyme systems in which the course is not complicated by side reactions. It may therefore be concluded that (1) the drop in viscosity is due to an enzyme present in the unheated gluten, (2) the enzyme reacts upon heated gluten as well as upon the unheated protein and (3) the rate of digestion of the heated gluten is the same as that of unheated gluten. It is realized that measurements of enzyme activity in terms of viscosity change are difficult but the consistency of the data and the fact stated previously, that the decreases in viscosity were greater than could be accounted for by changes in the unheated gluten alone, would seem to validate the conclusions in this case.

Effect of Heat on Gluten Dispersions

There was no detectable coagulation observed when either acetic acid or sodium salicylate (10%) dispersions of gluten were heated at 100°C for 5 to 30 minutes. A comparison of salt-precipitation curves for unheated dispersions and for dispersions heated 5 to 10 minutes suggested that no significant changes in solubility had occurred.

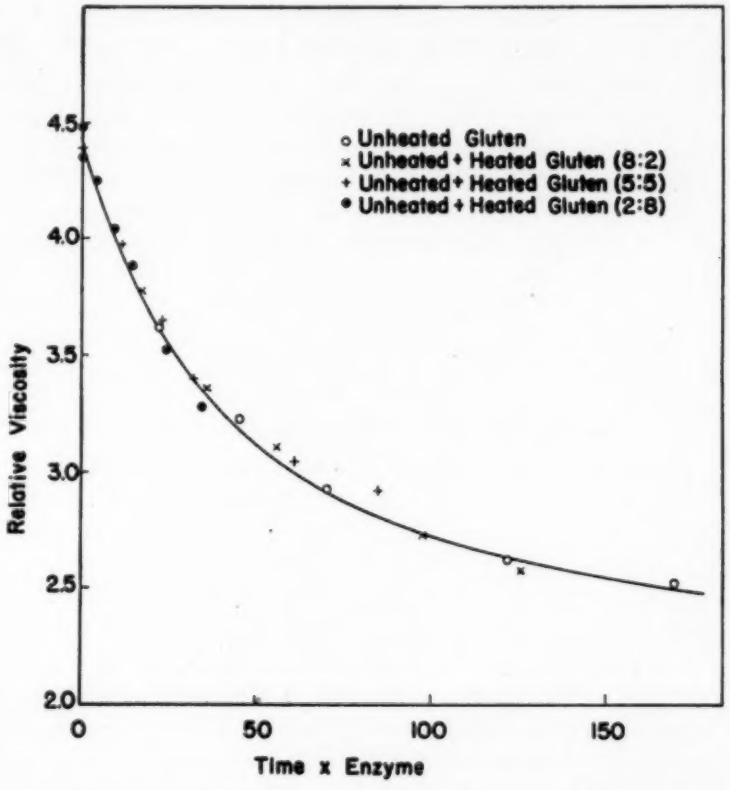

Fig. 2. The interchangeability of time and proteinase concentration (unheated gluten concentration) with regard to the change in relative viscosity of mixtures of heated and unheated gluten dispersions (see also Table I).

Gluten recovered from the heated solutions by salting out with magnesium sulfate possessed the kneadability and elasticity of the original protein. As shown above, heated dispersions of gluten were attacked by the gluten proteinase at rates equal to those shown by unheated dispersions. These combined observations strongly suggest that the heat treatment of gluten dispersions in dilute acetic acid or sodium salicylate does not cause extensive denaturation in the commonly understood definition. On the other hand, it is well known that subjecting gluten, not in dispersion, to similar conditions results in irreversible changes. It may be observed that short-time heat treatment caused variable, relatively small changes in the viscosity of the gluten solutions. In some cases, as in Figure 1, the viscosity was increased; in others, as in Figure 3, it was decreased. The significance of these findings is being investigated more fully.

Solubility of the Gluten Proteinase

Numerous attempts to prepare enzyme-free gluten by intensive washing of the gluten using successive disintegrations (Waring Blendor) in dilute salt solutions or distilled water, or by repeated re-solution in acetic acid and reprecipitation by salt or alkali addition, were not successful in removing the enzyme. The final products, after solution in dilute acetic acid, showed decreases in viscosity at rates comparable to the original glutens. In fractionations of washed gluten, the enzyme was found to follow the glutenin rather than the gliadin fraction. The apparent lack of solubility of the *gluten* proteinase in dilute salt solutions or water may differentiate it from the *flour* proteinase previously described (Hale, 1939) which is dispersed in these solvents.

Reversible Inactivation of Gluten Proteinase

Cook and Rose (1935; Rose and Cook, 1935) showed that dispersions of wheat gluten in 10% sodium salicylate were stable as indicated by viscosity and nonprotein nitrogen changes. Recently McCalla and Gralén (1942) found that the sedimentation behavior of a wheat gluten dispersion in sodium salicylate (8%) had not changed after standing for two months. In the present series of investigations, it was determined that the formol titration also did not change, at least for seven days at 25°C. The gluten proteinase is therefore inactive in 10% sodium salicylate. In order to determine whether such inactivation or inhibition was reversible, a sodium salicylate dispersion was dialyzed until the salicylate was completely removed. The precipitated gluten was then redispersed in acetic acid. A fall in viscosity with time, characteristic of the presence of the enzyme, was observed. However when the salicylate dispersion was heated at 100°C for 10 minutes prior to the dialysis, the viscosity of the subsequent acetic acid dispersion remained relatively constant. The enzyme is thus reversibly inactivated in 10% sodium salicylate. Permanent inactivation can be affected by heating the enzyme in the reversibly inactivated state.

Acidification of a salicylate gluten dispersion to pH 4.8 without precipitation was possible in the presence of 20% urea. Such a solution showed no drop in viscosity with time, whereas in the absence of the salicylate, a decrease in viscosity did occur. It is evident that the inhibition of the enzyme by the salicylate ion is specific and not due to the higher pH value (7.0) of sodium salicylate solutions.

The inactivity of the gluten proteinase in 10% sodium salicylate differentiates it from commercial papain, the activity of which was readily demonstrated in such an environment. Other papain-type proteinases were not investigated. One drawback in the use of salicy-

late dispersions for gluten investigations is indicated by these observations. Whenever, in subsequent manipulations, the salicylate is removed, the enzyme will again function as a modifying agent on the protein system.

Sosedov, Vakar and Drozdova (1940) have suggested the use of sodium salicylate (12%) dispersions of gluten as substrates for the assay of the enzyme activity of wheat samples. The present observations indicate that only proteolytic enzymes having properties differing from those of the gluten enzyme could be thus detected. Such enzymes apparently occur in germinated and probably in infested grains.

Effect of pH on Gluten Proteinase Activity

Because the original viscosities of the gluten solutions are themselves affected by pH, it is difficult to use changes in viscosity at different pH levels as a measure of the pH-enzyme activity relationship. Figure 3 shows that the viscosity of an acetic acid dispersion increases

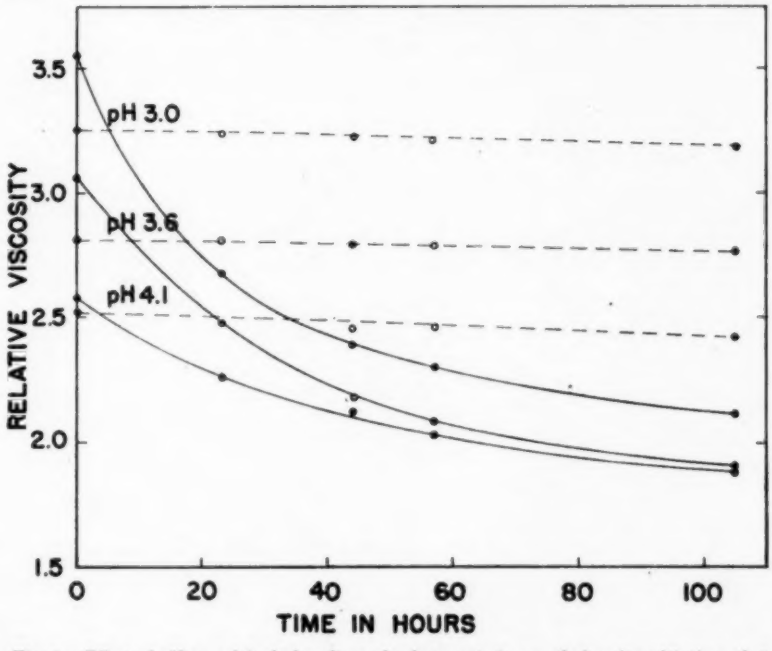

Fig. 3. Effect of pH on original viscosity and subsequent change of viscosity with time of gluten dispersions (4.82 mg N per ml) in acetic acid. pH 3.0 corresponds to 0.6 *N* acetic acid, pH 3.6 to 0.1 *N* acetic acid, and pH 4.1 to 0.025 *N* acetic acid approximately. The dotted lines represent the changes in viscosity of heated samples (10 min at 100°C).

as the pH is lowered from 4.1 to 3.0. Although the more viscous solutions are more drastically affected by the enzyme, this may be the result of the physical state of the protein, and does not necessarily indicate increasing rates of hydrolysis. However, formol titration

results show that the enzyme is most active at a comparatively low pH (3–4).

Effect of Reducing Agents on Gluten Dispersions Free From Enzyme Activity

Two systems were available for investigation: salicylate and heat-treated acetic acid dispersions. In the first case the enzyme was temporarily inactivated because of its environment; in the second case it had been permanently inactivated by heat. In both cases, immediate and drastic decreases in viscosity followed the addition of dilute solutions of cysteine, monothioglycol, sodium bisulfite, and potassium cyanide. That the decrease in viscosity could not be attributed to enzymatic activity was definitely indicated by the constancy of the formol titration. Some of the results are indicated in Tables II and III and Figure 4.

TABLE II

COMPARISON OF ENZYMATIC CHANGE IN UNHEATED GLUTEN DISPERSION WITH EFFECT OF MONOTHIOGLYCOL ON HEATED GLUTEN DISPERSION AT 25°C[1]

Dispersion	Relative viscosity		Formol titration[2]	
	1 hr	42 hrs	0 hr	42 hrs
Gluten	4.0	2.8	1.3	2.0
Heated gluten[3]	4.6	4.6	1.3	1.3
Heated gluten + 0.01*M* monothioglycol	3.9	2.1	1.1[4]	1.1

[1] All solutions contained 6.3 mg N per ml in 0.1 *N* acetic acid, pH 3.7.
[2] As ml 0.1 *N* NaOH required per g of gluten.
[3] Heated 5 minutes at 100°C.
[4] The presence of thioglycol results in a decrease in titratable acid. Thioglycol has an acidic SH group which reacts with formaldehyde, releasing alkali.

TABLE III

EFFECT OF SODIUM BISULFITE (0.004*M*) ON A HEATED GLUTEN DISPERSION IN 0.01*N* ACETIC ACID AT 40°C[1]

Time after addition	Relative viscosity	Time after addition	Relative viscosity
min		*min*	
0	3.0	10	2.53
2	2.86	14	2.49
4	2.72	31	2.34
6	2.63	44	2.29
8	2.59		

[1] In contrast, sodium bisulfate in the same concentration caused no similar progressive decrease in viscosity.

Effect of Reducing Agents on the Gluten Proteinase

As shown in Table IV, thioglycol caused a slight increase in the rate of hydrolysis of gluten by its proteinase in 0.1*N* acetic acid. This

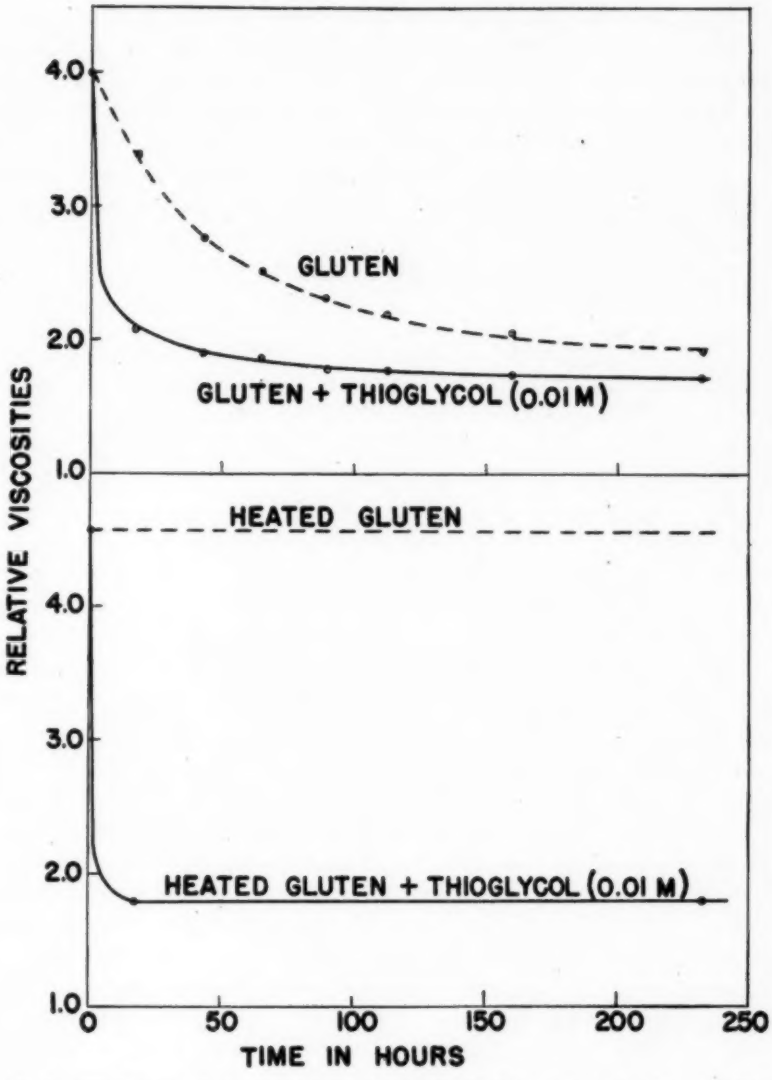

Fig. 4. Effects of a reducing agent (monothioglycol, 0.01 *M*) on unheated and heated (10 min at 100°C) gluten dispersions (6.3 mg N per ml) in acetic acid (0.1 *N*) at 25°C.

result was observed also with the other reducing agents previously mentioned. The activation with all reducing agents was relatively unimportant in degree.

Discussion

The occurrence of proteolytic activity in flours is now accepted, and the properties of a flour proteinase have been described by Balls and

TABLE IV

EFFECT OF MONOTHIOGLYCOL AS ACTIVATOR FOR GLUTEN PROTEINASE

Dispersion	Formol titration[2]		Nonprotein nitrogen[3]	
	1 hr	237 hrs	18 hrs	240 hrs
Gluten[1]	1.3	3.8	2.8	11.3
Gluten + thioglycol (0.01*M*)	1.2	4.6	3.5	12.7

[1] In 0.1 *N* acetic acid, pH 3.7, contained 6.3 mg N per ml final concentration.
[2] As ml of 0.1 *N* NaOH per g of gluten.
[3] As percentage of total nitrogen.

Hale (1936a), Jørgensen (1936), Hale (1939) and others. That a proteinase is closely associated with gluten and cannot be washed out has not, however, previously been reported. It remains to be determined whether the gluten enzyme differs in other respects from the flour proteinase previously described.

The importance of the gluten enzyme in baking technology is problematical. The activity is so weak, even after activation, that it is doubtful whether it can play an important role. That the enzyme is more active in the pH range 2 to 4, than in that ordinarily encountered in doughs, tends to confirm such an interpretation.

Jørgensen (1936) and Balls and Hale (1936a) (see Hale, 1939, also) suggested independently that the remarkable softening effect of reducing agents on doughs and glutens could be attributed to their activation of native proteinases, although the latter authors pointed out that a direct chemical effect on the gluten could not be ruled out. The present evidence shows that, in addition to their activation of native proteinases, reducing substances exert profound changes on gluten in dispersion even though (1) the gluten proteinases have been destroyed by heat or (2) the gluten proteinases have been entirely inactivated in the presence of 10% sodium salicylate. It may be concluded, in confirmation of the suggestion of Ford and Maiden (1938) (see also Balls and Hale, 1936), that the effect of reducing agents in glutens and flours is primarily a chemical one on the protein molecule and only secondarily that of activating the native proteinases present. The possibility that the increased digestibility of gluten by papain-type enzymes in the presence of reducing agents may be due in part to the altered gluten, rather than to a specific activation of the enzyme, deserves consideration.

Summary

Gluten dispersions in dilute acetic acid may be stabilized by heating for 5 to 10 minutes at 100°C. The solubility characteristics of the

gluten proteins are not appreciably altered, but an enzyme (proteinase) responsible for the instability is destroyed. The proteinase is inactivated or inhibited in 10% sodium salicylate but activity is restored and the proteinase-induced hydrolysis continues if the salicylate is removed. Reducing agents increase the enzymatic hydrolysis slightly but they also drastically reduce the viscosity of gluten freed from enzyme activity by heat or by the presence of 10% sodium salicylate. It is concluded that the primary effect of reducing agents on *glutens* is a chemical one upon the proteins and only secondarily that of enzyme activation, and it is suggested that a similar explanation may be applied to the effect of reducing agents, but not necessarily oxidizing agents, on *doughs*.

Acknowledgment

The authors are indebted to Dr. Hans Lineweaver for many helpful suggestions and for calling their attention to the relationships implied in Figure 2. The Carbide and Carbon Corporation supplied a generous sample of monothioglycol.

Literature Cited

Balls, A. K., and Hale, W. S.
1936a Proteolytic enzymes of flour. Cereal Chem. **13**: 54–60.
1936b Further studies on the activity of proteinase in flour. Cereal Chem. **13**: 656–664.
Blish, M. J., and Sandstedt, R. M.
1925 Glutenin—a simple method for its preparation and direct quantitative determination. Cereal Chem. **2**: 57–67.
1929 The nature and identity of wheat glutenin. J. Biol. Chem. **85**: 195–206.
Bodansky, O.
1937 The use of different measures of reaction velocity in the study of the kinetics of biochemical reactions. J. Biol. Chem. **120**: 555–574.
Cook, W. H., and Rose, R. C.
1935 Hydrolysis of gluten induced by the solvent. Can. J. Research **12**: 248–252.
Ford, W. P., and Maiden, A. M.
1938 Effects in dough of glutathione and papain. J. Soc. Chem. Ind. **57**: 278–281.
Hale, W. S.
1939 The proteinase in wheat flour. Cereal Chem. **16**: 695–702.
Jørgensen, H.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. Cereal Chem. **13**: 346–355.
Larmour, R. K., and Sallans, H. R.
1932 A comparison of glutenin and gliadin prepared from one flour by various methods. Can. J. Research **6**: 38–53.
McCalla, A. G., and Gralén, N.
1942 Ultracentrifuge and diffusion studies on gluten. Can. J. Research **C20**: 130–159.
Rose, R. C., and Cook, W. H.
1935 Viscosity of gluten dispersed in alkali, acid and neutral solvents. Can. J. Research **12**: 63–81.
Sosedov, N. I., Vakar, A. B., and Drozdova, Z. B.
1940 Determination of proteolytic enzyme activity and wheat protein resistance to enzymes. Biokhimiya **5**: 464–474; through Chem. Absts. **35**: 4859 (1940).

THE COMPLEMENTARY BAKING PROPERTIES OF MINNESOTA SPRING AND WINTER WHEAT VARIETIES [1]

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In evaluating the quality of new wheat varieties produced by plant breeders, laboratory studies are carried out on individual samples and the results compared with those obtained with varieties of established commercial value grown under the same environmental conditions. Baking tests using different formulas and procedures are employed which under ideal conditions are designed not only to reveal the inherent strength of the sample but also to secure measures of mixing, length of fermentation, and oxidation requirements necessary to obtain an optimum loaf. In a recent paper Geddes (1941) has expressed the view that a new variety should give satisfactory and, preferably, optimum results in milling and baking when processed in the same manner as the "standard variety," if it is to meet with ready acceptance by the trade. Definite inferiority in any one important quality characteristic is generally regarded as sufficient to render a variety unsuitable for release even though it is superior to the standard in certain particulars. In other words the new wheat must yield a flour which is "well-balanced" in regard to the various attributes of quality.

Not infrequently, hybrids of superior agronomic characteristics are produced which tests indicate to be of lower strength or to possess certain baking properties which depart somewhat from those of the accepted standard. Under such circumstances it is difficult indeed to reach a decision. Commercially, such a variety would show considerable variation in characteristics as a result of being grown under various soil and climatic conditions and would normally be milled in mixtures with other varieties or classes of wheat. It may be argued with considerable justification that it would be to the miller's advantage to have wheat varieties available which differ in certain inherent quality characteristics such as mixing requirements and dough handling properties. By judicious wheat selection and blending he would be in a better position to produce uniform flour of the desired characteristics, for the various classes of trade which he serves.

The question arises as to whether laboratory tests conducted on pure varieties are fully adequate indices of the commercial value. Minor variations from the standard in baking characteristics may not

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be discernible in blends. Moreover, different varieties when blended may complement each other, thereby producing a flour of superior baking properties to that which would be predicted from a knowledge of the characteristics of the individual components. There is little information in the literature on this subject although the writings of English cereal chemists indicate that there are considerable differences in the compatibility of different world wheats in blends. Larmour (1931) included a blend formula in a study of the relation between wheat protein content and loaf volume of the flours milled therefrom. In an effort to secure more useful information regarding the carrying power of Canadian wheats when used in such representative blends as European millers and bakers employed, Geddes (1935) introduced a blend formula in the routine testing of average samples of the various grades of the Western Canadian wheat crop. In both these studies, the flour under investigation constituted 50% of the blend. In view of the almost limitless number of blends which may be prepared and the difficulties involved in interpreting the results, it is not at all surprising that little work of this nature has been reported.

The authors were led to undertake the preliminary series of blend baking tests reported here in an effort to secure additional information which might be of value in reaching a decision as to whether a new rust-resistant, high-yielding hard red spring wheat variety named Merit should be distributed. Tests in several laboratories indicated that this variety was of low strength but there was a question as to the adequacy of the methods since the application of several baking formulas by one laboratory indicated that the variety possessed relatively higher inherent strength when baked by a commercial-type formula. It seemed that its inherent strength might better be revealed by measuring its carrying power for Minnesota hard winter wheat flours in comparison with other spring wheats as controls. While these blending studies are limited in scope and are of a preliminary nature, they are presented as an indication of the possible value of this technique in variety studies.

Experimental

Composite samples of three experimentally milled spring wheat flours representing Merit (strain 3), Thatcher and a Thatcher back cross were each blended with 25, 50, and 75% of a winter wheat flour, experimentally milled from a Minnesota-grown Minturki back cross. Similar blends were prepared with two of the spring wheat flours, namely Thatcher and the Thatcher back cross. The protein contents of the flours, expressed on a 15% moisture basis were: Thatcher 12.9%, Merit-3 13.1%, Thatcher B.C. 14.8%, and Minturki B.C. 12.7%. The original flours and blends were baked by the regular A.A.C.C.

formula, as outlined in Cereal Laboratory Methods (4th ed., 1941), but with the addition of 0.001% potassium bromate. A differential baking procedure, involving two mixing times of 2 and 4 minutes in the Hobart-Swanson mixer, and two fermentation periods of 2 and 3 hours, was used. Two hundred grams of flour was mixed each time and two 150-gram doughs, one for each fermentation time, scaled off immediately after mixing. The doughs were punched in the National dough sheeter and baked in the low-form tins described by Markley (1940). All bakings were made in duplicate on different days with the exception of the blends of the two spring-wheat flours for which insufficient material was available.

The mean loaf volumes obtained are represented graphically in Figure 1. It is evident that the unblended flours as a group differ appreciably not only in general strength but also in the extent and nature of their responses to the different mixing and fermentation times employed. Thus the mean loaf volumes for all treatments combined are 800 cc for Thatcher, 789 cc for Thatcher B.C., 684 cc for Merit-3, and 758 cc for Minturki B.C. All the varieties gave their lowest volumes with 4 minutes of mixing and 3 hours of fermentation, but of the spring wheat flours, Merit showed the least variation with treatment and the Thatcher back cross the greatest. The winter wheat Minturki B.C. gave very similar loaf volumes for all other treatments, although a short mixing and fermentation requirement is suggested by the fact that it gave its greatest volume with 2 minutes of mixing and 2 hours of fermentation. In contrast, Thatcher required 4 minutes of mixing and 2 hours fermentation to produce its best loaf. Thatcher B.C. gave an optimum loaf volume with 2 minutes of mixing and 2 hours of fermentation, indicating that this wheat is more similar to the winter wheat variety than Thatcher in its mixing and fermentation requirements. As Merit yielded its best loaf with 2 minutes of mixing and 3 hours of fermentation, this variety appears to be characterized by requiring a longer fermentation time than any of the others.

Markley (1937) has emphasized the importance of employing a differential baking test which will give a rough measure of the mixing, fermentation, and oxidizing requirements of the flours under study. As exemplified by the present results, the application of this principle has proved very useful in variety testing at this institution. While more extensive studies employing various mixing and fermentation times are obviously necessary to establish definitely the optimum requirements for a given flour, the differentials employed are sufficient to show major departures in these respects from the ideal which is being sought. This information is always considered in conjunction with the absolute volumes which are taken as a measure of strength.

Turning now to a consideration of the loaf data obtained for the blends, these do not appear to bear a strictly additive relationship to the volumes of the two components; while the extent of the departure varies somewhat with the varieties, its magnitude and also its direction

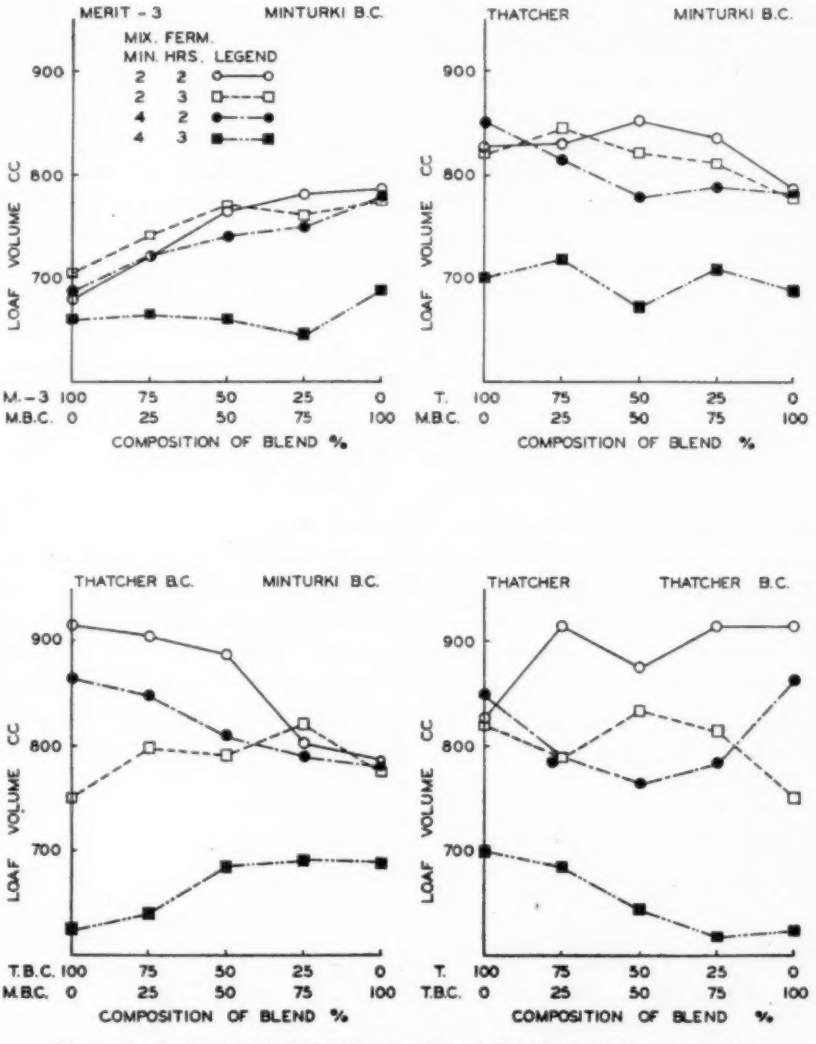

Fig. 1. Loaf volumes of individual flours and blends for different baking treatments.

are more influenced by the baking procedure. Thus the average difference between the observed and calculated volumes of the 25%, 50%, and 75% blends was +31, +14, and +23 cc for Thatcher, Thatcher B.C., and Merit-3, respectively, when baked after 2 minutes of mixing

and 2 hours of fermentation. For 2 minutes of mixing and 3 hours of fermentation the corresponding divergences were +27, +40, and +18 cc; for 4 minutes mixing and 2 hours of fermentation −23, −8, and +2 cc, respectively; and for 4 minutes of mixing and 3 hours of fermentation +5, +15, and −18 cc, respectively. The question arises as to whether or not these differences between the observed and expected values represent real departures from linearity. Unfortunately, there are not sufficient data available for any one blend and baking procedure to determine with any degree of precision whether curves would fit these data better than straight lines. However, several of the differences between the observed and calculated values exceeded twice the standard error for the particular baking procedure employed. In other cases, such as the Thatcher and Minturki B.C. blends mixed 2 minutes and fermented 3 hours, the divergence is positive for all the mixtures, although no individual blend gave a significantly greater loaf volume than the calculated value. While it cannot be stated that complementary effects have been demonstrated the data are suggestive.

It is of particular interest to note the influence of the baking method on the direction and magnitude of the apparent departure of the loaf volumes of the blends from purely additive functions of the volumes of the component flours. These results indicate that the demonstration of complementary effects will depend both upon the properties of the wheats or flours under study and the baking methods employed. Where a strong wheat or flour exhibits "carrying power" or "reserve strength" for a weaker one, it is probable that the baking procedure employed is more nearly optimum for the blends than it is for either of the two components. On the other hand, where the baking method is more nearly optimum for the components than for the blends, the blend values would be expected to be less than those calculated on an additive basis.

These results must be regarded as preliminary in nature and are presented merely as an indication of the utility of employing differential baking procedures in evaluating wheat and flour strength and blending properties.

Summary

Flours representing three spring wheat varieties, Thatcher, a Thatcher back cross, and Merit, and one winter wheat variety, Minturki back cross, were baked by a differential procedure employing mixing times of 2 and 4 minutes and fermentation times of 2 and 3 hours. Flour blends containing 25%, 50%, and 75% of the winter wheat flour were made with each of the spring wheat flours and baked by the same procedures.

The relative strength of the unblended flours, arranged in decreasing order, as indicated by the average loaf volumes for all treatments, was: Thatcher, Thatcher back cross, Minturki back cross, and Merit, but the varieties differed markedly in their response to the different baking procedures. Merit and Minturki back cross exhibited the least differences between the various treatments and Thatcher back cross the greatest. The use of differential baking treatments in variety testing is valuable in providing an indication of the mixing and fermentation requirements.

Loaf volumes of the blends did not appear to be proportional to the calculated values based on the volumes of the component flours, the direction and magnitude of the divergences depending to a large extent on the baking procedure.

The studies must be regarded as preliminary in nature but are indicative of the utility of differential baking procedures in evaluating wheat and flour strength and blending properties.

Literature Cited

Geddes, W. F.
1935 Dominion Grain Research Laboratory, Winnipeg, Manitoba. Ninth Annual Report, Department of Trade and Commerce, Ottawa, Canada.
1941 Objectives in breeding for improved quality in hard wheat. J. Am. Soc. Agron. **33**: 490–503.
Larmour, R. K.
1931 The relation of wheat protein to baking quality. II. Saskatchewan hard red spring wheat crop of 1929. Cereal Chem. **8**: 179–189.
Markley, M. C.
1937 The scientific basis for experimental baking tests. Cereal Chem. **14**: 834–840.
1940 Test baking pan design. Cereal Chem. **17**: 387–392.

THIAMIN IN PRODUCTS OF COMMERCIAL RICE MILLING [1]

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Several publications (Nordgren and Andrews, 1941; Conner and Straub, 1941) have reported the thiamin contents of cereal grains other than rice. In those papers the importance of cereals as a dietary source of thiamin has been emphasized.

This investigation deals with the thiamin content of rice, which is the main cereal crop in the Orient and an increasingly important one in the southern states. It has been known to be a good source of the

[1] Research paper No. 746 Journal Series, University of Arkansas, published with the approval of the Director of the Arkansas Agricultural Experiment Station.

anti-beri beri vitamin since the experiments of Eijkman (1889) and of Grijns (1901). However, no intensive investigation has been made of the fate of thiamin during the commercial milling process.

As the first step in milling (Reed and Liepsner, 1917) hulling stones remove the hulls from the previously cleaned rough rice, leaving brown rice and the hulls. By friction, part of the bran layer from the outside kernel and most of the germ are removed from the brown rice with the aid of hullers. First-break huller rice is obtained with first-break bran as a by-product. The kernels are next conveyed to a second set of hullers, which remove more bran and leave second-break huller rice and second-break bran. In some mills, in addition, a pearling cone is also used in this step, which gives pearling-cone rice and a by-product which is called pearling-cone polish or pearling-cone meal.

In order to remove more of the inner coat and to give the rice a smoother finish, it is removed to the so-called brush. The products here obtained are brush rice and brush polish.

The rice milling, then, consists of the removal of hulls and outer and inner-bran layers in order to obtain a product of suitable appearance and attractiveness. The milled, clean rice is now separated into different grades. The unbroken kernels are known as head rice, which is given a high finish with the aid of a small amount of glucose and talc. The broken kernels are known as second heads, screenings, and brewer's rice.

Experimental

Samples of the same mill lots, gathered at suitable time intervals, were obtained during the milling season of 1941–42 from various rice varieties.[2] Others were taken from the same variety of different mill lots in order to find out possible differences due to the milling or to other environmental factors. Samples of the following varieties and mill lots were investigated: Supreme Blue Rose, medium grain variety, mill lots 551 and 606; Early Prolific, medium grain variety, mill lots 569 and 663; Fortuna, long grain variety, mill lot 635; Lady Wright, long grain variety, mill lot 768; and Improved Blue Rose, medium grain variety, mill lot 778. All of the samples were from rice varieties grown in fields which were not fertilized and which were irrigated by well water. The varieties were grown in Arkansas except Improved Blue Rose, which was grown in a Louisiana field.

The samples were analyzed for thiamin hydrochloride by the thiochrome method, with the adaptation of the Hennesy and Cerecedo procedure (1939).[3] The method as used in our laboratory is briefly as follows: Samples of 1 to 15g (depending on vitamin content) are sus-

[2] Samples were obtained from the Walton Mill, Inc., Stuttgart, Arkansas.
[3] As carried out in the laboratories of Merck and Co., Rahway, N. J.

pended in 75 ml of 0.1*N* H_2SO_4, autoclaved in a pressure cooker for 20 minutes at 15 pounds pressure, cooled, adjusted to pH 4.0 to 4.5 with 2*N* sodium acetate solution with brom cresol green as indicator. Five ml of 10% takadiastase solution is added, mixed, and incubated at 50°C for at least 1½ hours. After incubation the mixture is cooled, made up to 100 ml, and filtered. Aliquots of the clear extracts are used for the thiamin determination.

Thiamin occurs in two forms, in the free form and in the bound form as a pyrophosphoric ester or cocarboxylase. The bound form is changed to the free form with the aid of an enzymatic hydrolysis, and the two forms make up the total thiamin of the sample. A test without enzymatic hydrolysis gives the amount present in the free form and the difference between this and the total represents the thiamin present in the bound form. Values for both free and bound thiamin have been determined for one set of samples. The thiamin content is expressed as micrograms per gram of dry material.

Results

The data in Table I show that rough rice or paddy and whole brown rice contained about 3 μg of thiamin (approximately 1 International Unit) per gram, while brown rice contained slightly more thiamin than rough rice. During the milling or bleaching process a considerable part of the thiamin is removed. Whole rice of Supreme Blue Rose variety (mill lot 551) had a thiamin content of 2.58 μg per gram of dry matter, which was reduced to 0.74 μg per gram in milling—a loss of 71.3%. In the second mill lot (606) of the same variety 81.7% was lost and only 18.3% remained in the polished rice. Similar losses have been found for the other varieties and mill lots.

Early Prolific (mill lot 569) lost 81.5%, and 80.6% was removed in the second mill lot 663. Fortuna lost 82.3% and the losses in Lady Wright and Improved Blue Rose were 86.5% and 77.8%, respectively.

As an average of the seven mill lots investigated 19.8% of the thiamin, originally present in the whole brown rice, remained in the end product sold for human consumption and 80.2% had been removed.

Small differences were found in the thiamin contents of brown rice of different varieties and even in those of different mill lots of the same variety. This may be due to the fact that milling is not a standard procedure, since readjustment of the huller blades may take place during the milling process. However, rice from a different mill lot might have grown under slightly different field conditions even in the same field and this might be responsible for the observed difference. Further studies are under way to reveal the influence of variety. In the process of milling, most of the thiamin is removed by the hullers (first and

TABLE I

THIAMIN CONTENT (TOTAL, FREE, AND BOUND) IN PRODUCTS OF COMMERCIAL RICE MILLING[1]

Products	Variety[2] and mill lot										
	Supreme Blue Rose					Early Prolific			Fortuna	Lady Wright	Improved Blue Rose[4]
	551					606	569	663	635	768	778
	Total	Bound		Free		Total	Total	Total	Total	Total	Total
	μg/g	μg/g	%	μg/g	%	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Paddy or rough rice	2.60	0.77	29.6	1.83	70.4	3.00	3.03	3.00	2.79	3.00	3.07
From milling or bleaching process:											
Whole brown rice	2.58	1.15	44.6	1.43	55.4	3.01	3.51	2.99	3.05	3.00	3.00
First break huller rice	0.88	0.08	9.0	0.80	91.0	0.97	0.92	0.76	0.84	1.04	0.95
Second break huller rice	0.87	0.32	36.8	0.56	63.2	0.85	0.96	0.65	0.77	0.73	0.95
Pearling cone rice	0.83	0.31	37.3	0.52	62.7	0.91	0.94	0.75	[3]	[3]	0.70
Brush rice	0.80	0.34	42.5	0.46	57.5	0.53	0.79	0.67	0.58	0.45	0.66
Finished, clean, polished rice:											
Head rice	0.74	0.16	21.6	0.60	78.4	0.55	0.65	0.58	0.54	0.49	0.63
Second head rice	0.46	0.12	26.1	0.34	73.9	0.51	0.55	0.49	0.47	0.46	0.58
Screenings	0.91	0.43	47.2	0.48	52.8	0.75	0.86	0.76	0.66	0.77	0.80
Brewers rice	1.35	0.35	26.0	1.00	74.0	1.34	1.20	1.42	1.67	1.57	1.30
Rice by-products:											
Hulls	1.23	0.53	43.1	0.70	56.9	0.84	1.35	1.11	0.93	1.33	1.00
First break bran	33.00	8.10	24.5	24.90	75.5	33.60	32.20	23.90	21.50	26.60	20.50
Second break bran	24.50	3.30	13.4	21.20	86.6	16.50	16.30	20.30	11.80	17.60	18.00
Pearling cone polish	26.50	5.30	20.8	21.20	79.2	25.70	22.00	19.40	[3]	[3]	20.80
Brush polish	27.90	3.10	11.0	24.80	89.0	26.70	21.30	20.00	15.00	19.40	16.40

[1] Obtained from one mill.
[2] All varieties are from fields which were not fertilized and were irrigated by well water.
[3] Pearling cones are not used in long grain varieties.
[4] Grown in Louisiana.

second break) which indicates that the thiamin is mainly present in the outer bran layers and in the germ.

Of the finished, clean product, the head rice contained somewhat more thiamin than the second head. The average thiamin content of the head rice was 0.60 μg per gram and of the second head 0.50 μg per gram. The screenings had an average content of 0.79 and the brewer's rice 1.40 μg per gram. The probable reason for this is that screenings and brewer's rice consist of a great many broken pieces, among them particles of rice embryo, which is a rich source of thiamin and which is mainly present in the bran. These products are good sources of thiamin for feed for livestock and in making beverages.

Of the by-products, rice hulls contained an average of 1.11 μg per gram of thiamin. Since these have at present little or no commercial value it is suggested that large quantities of hulls if properly extracted might yield considerable amounts of thiamin.

Rice bran and rice polish were found to be excellent sources of thiamin. Rice bran (first break) contained from 20.5 to 33 μg per gram. Second-break bran contained less.

Rice polish (brush) contained less thiamin than rice bran; the range was from 15 to 27.9 μg per gram, but it must still be considered an excellent source. About the same range was found in the cone polish. Since pearling cones were not used for the long-grain kernels, no data on cone polish could be obtained for the long-grain varieties Fortuna and Lady Wright.

Free and bound thiamin have been determined in all samples of Supreme Blue Rose, mill lot 551. From Table I it is apparent that thiamin mainly occurs in the free form; the range was from 91% in first-break huller rice to 52.8% in screenings. In the by-products, bran and polish, from 75% to 90% was found to be present in the free form, which is of interest since it is not known definitely whether the physiological functions in the body of both forms of thiamin are the same.

Included in this report are preliminary data obtained on thiamin content of parboiled milled rice, nonparboiled milled rice, and under-milled rice.

Parboiled rice may be rough rice which is parboiled prior to milling. The process of parboiling consists essentially of soaking rough rice in water, followed by draining of the water, steaming of the rice, and drying. The hypothesis is that the water-soluble vitamins diffuse from the outer layers of the kernel to the inner layers, and after the process of milling more of these water-soluble nutrients are retained in the kernel.

The results of this assay are given in Table II, from which it is evident that the thiamin content in milled parboiled rice is higher than

TABLE II

THIAMIN CONTENT (IN μG PER GRAM) OF MILLED PARBOILED AND MILLED NON-PARBOILED RICE, MILLED AND UNDER-MILLED RICE

Variety	Treatment	μg/g
Nira [1]	milled, parboiled	1.35
Calora [2]	milled, parboiled	1.61
Indian [3]	milled, parboiled	1.74
Nira [1]	milled, non-parboiled	.59
Lady Wright [3]	under-milled	1.22
Lady Wright [3]	milled	.57
Supreme Blue Rose [2]	under-milled	.92
Supreme Blue Rose [2]	milled	.65

[1] Obtained through the courtesy of Dr. C. R. Adair, Associate Agronomist, U. S. Department of Agriculture, Bureau of Plant Industry, Rice Branch Experiment Station, Stuttgart, Arkansas.
[2] Obtained through the courtesy of the Rice Growers Association of California, Sacramento, California.
[3] Obtained through the courtesy of the Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas.

in milled nonparboiled rice. A value of 1.35 μg per gram was obtained for the former and of 0.59 μg per gram for the latter. These samples were of the same lot and belonged to the Nira variety. Another sample of Indian parboiled rice contained 1.70 μg per gram, and a sample of Calora from California contained 1.61 μg per gram. Experiments with parboiled rice are now being continued on an extensive scale in order to find out the optimum conditions of treatment for the maximum retention of the water-soluble vitamins and other water-soluble nutrients. In under-milled rice, more of the bran layers are left on the kernel, which leave more of the vitamins and other nutrients in the finished product. The thiamin content of under-milled rice is higher than of the milled rice as is shown in Table II. An under-milled sample of Lady Wright variety contained 1.22 μg per gram compared with 0.57 μg for the sample of ordinary milled rice, or more than twice as much thiamin was found in the under-milled rice.

Summary

A study has been made of the thiamin content in products of commercial rice milling, with the following results:

Rough rice or paddy and whole brown rice contained about 3 μg of thiamin or approximately one International Unit per gram of dry material. Whole brown rice contained slightly more thiamin than rough rice.

An average of 80% of the thiamin was lost during the milling process.

From 50% to 90% of the thiamin was found to be present in the free form; this range was from 76% to 90% for the by-products rice bran and rice polish.

Of the finished, clean products, the end product head rice (sold for human consumption) contained an average of 0.60 μg and second head 0.50 μg per gram of dry matter. Screenings and brewer's rice contained 0.79 μg and 1.40 μg thiamin, respectively.

Of the by-products, hulls contained 1.11 μg thiamin per gram of dry matter, rice bran contained from 20.5 to 33 μg, and rice polish from 15 to 27.9 μg.

Three different samples of milled parboiled rice (prepared in three different localities) contained 1.35, 1.61, and 1.74 μg thiamin per gram of dry material. Two different samples of under-milled rice contained 1.22 and 0.92 compared with 0.57 and 0.65 μg in milled rice.

Acknowledgment

The author acknowledges with thanks the valuable assistance of Mr. C. R. Walton of the Walton Rice Mill, Inc., Stuttgart, Arkansas, for furnishing the rice mill samples.

Literature Cited

Conner, R. T., and Straub, G. J.
1941 The thiamin and riboflavin contents of wheat and corn. Cereal Chem. **18**: 671–677.
Eijkman, C.
1889 Jaarverslag v. h. Lab. voor Path, Anat. en Bacteriologie.
Grijns, G.
1901 Over Polyneuritis Gallinarum Geneesk. Tijdschrift v. Ned. Indie **41**: 3.
Hennessy, D. J., and Cerecedo, L. R.
1939 The determination of free and phosphorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. **61**: 179–183.
Nordgren, R., and Andrews, J. S.
1941 The thiamin content of cereal grains. Cereal Chem. **18**: 802–811.
Reed, J. B., and Liepsner, F. W.
1917 The by-products of rice milling. U. S. Dept. Agr. Bul. 570.

A STUDY OF SOME OF THE VITAMIN B COMPLEX FACTORS IN MALTED AND UNMALTED BARLEY AND WHEAT OF THE 1941 CROP

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The primary purpose of the studies reported here was to determine the relation between unmalted and malted wheat and barley with respect to the B-complex vitamins—thiamin, riboflavin, pantothenic acid, and nicotinic acid—for which reliable analytical methods have been established.

Experimental

Seven samples of barley of widely different origin and their respective malts were obtained from malt houses. All of these were standard commercial malts. Five samples of wheat representing widely different types were obtained. A commercial malt from one of these was obtained and the other four samples were laboratory malted at approximately 15°C for six days. These samples were dried and kilned under conditions as nearly as possible like those in large-scale malting.

Thiamin determinations were made according to the thiochrome method outlined in the *Cereal Laboratory Methods* (1941) with the exception that the enzyme polidase was used in the incubation of the samples. The riboflavin assay was made with the microbiological method of Snell and Strong (1939) using the *Lactobaccilus casei-ε* organism and an enzyme treatment for preparation of the sample as outlined by Andrews, Boyd, and Terry (1942b). Pantothenic acid was determined in a similar manner with the same organism and the procedure outlined by Pennington, Snell, and Williams (1940).

Nicotinic acid was assayed microbiologically by means of the organism *Lactobacillus arabinosus* 17–5 and the procedure described by Snell and Wright (1941).

Because the moisture contents of grain and malt are considerably different, all results were calculated on the dry-matter basis. On the dry-matter basis there is an 11% to 14% substance loss from grain to malt in usual malting practice. If vitamin content in malted grains is the same as in the original grain or shows a loss no greater than the substance loss, then one could assume that no great amount of the vitamin material has been consumed, destroyed, synthesized, or released. This work was done on finished malt; no data are given on the progress of the vitamin content during the various stages of malting.

Results

In Table I are presented the results from testing all samples for the four B complex factors: thiamin, riboflavin, nicotinic acid, and pantothenic acid.

TABLE I

B-Complex Values Found in Malted and Unmalted Grains

(All values expressed in μg per gram, dry matter basis)

Origin	Thiamin		Riboflavin		Nicotinic acid		Pantothenic acid	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Barley								
So. Dakota	4.96	4.29	0.78	3.52	90.2	86.8	5.30	5.85
So. Minn.	5.04	4.65	1.12	3.35	98.1	105.4	6.20	8.30
Choice Iowa	6.52	5.80	1.08	3.20	89.6	97.5	5.13	5.71
Fancy Ill.	4.91	5.15	1.14	2.76	83.0	95.4	4.01	4.63
Special	6.32	5.94	1.00	2.90	89.4	101.0	4.31	6.03
Wisconsin	5.99	5.27	1.06	3.04	85.1	90.8	3.95	5.24
Hannchen	5.38	5.81	0.97	2.83	69.0	82.1	2.86	4.75
Mean	5.59	5.27	1.02	3.09	86.3	94.1	4.5	5.8
Wheat								
White	4.58	4.49	0.83	3.67	52.7	61.5	9.44	9.76
White, Mich.	6.38	5.85	1.10	2.88	62.7	71.9	9.19	9.20
Spring	5.56	5.46	1.12	2.27	49.3	65.7	6.91	9.82
Red Winter	5.22	4.58	0.71	3.08	62.3	66.3	7.23	7.92
Winter	6.64	4.70	0.77	2.65	66.1	71.4	7.05	8.64
Mean	5.68	5.02	0.91	2.91	58.6	67.3	7.9	9.0

The thiamin assay gave values of 5.6 and 5.7 μg per gram, respectively, for barley and wheat and 5.3 and 5.0 μg per gram, respectively, for malted barley and malted wheat. These differences between the malted and unmalted grains are not highly significant, showing an average decrease in most cases in the malted grain slightly greater than the substance loss of the grain during malting. The thia-

min found in these samples compares favorably with average values of 5.13 μg per gram for barley and 4.98 μg per gram for malt found by Laufer, Brenner, and Laufer (1940); 6.85 μg per gram for whole wheat found by Hoffman, Schweitzer, and Dalby (1940); 6.2 and 5.6 μg per gram as average values for barley and wheat found by Schultz, Atkin and Frey (1941); 7.1 μg per gram for hard wheat and 6.1 μg per gram for soft wheat found by Downs and Cathcart (1941); 5.03 μg per gram for hard wheat and 3.5 μg per gram for soft wheat found by Conner and Straub (1941); 4.86–5.28 μg per gram for barley found by Conner and Straub (1941), and 5.8 and 6.5 μg per gram for wheat and barley, respectively, reported by Nordgren and Andrews (1941).

The riboflavin values show a highly significant increase in the malted grain over the unmalted. The average content of the barley was 1.02 μg per gram and for the barley malt 3.09 μg per gram. The assay on wheat was 0.91 μg per gram and the wheat malt 2.91 μg per gram. The average results on both grains showed an apparent increase of approximately 175% due to the malting process. These values for the unmalted grain compare favorably with the levels of 0.89–2.03 μg per gram reported by Conner and Straub (1941) and 1.21 μg per gram for barley and 1.17 μg per gram for wheat reported by Andrews, Boyd, and Terry (1942a).

The nicotinic acid results show an increase in the malted samples, the difference being of low magnitude although in all samples except one the malted sample showed the higher values. The assay value for barley and wheat was 86.3 and 58.6 μg per gram, respectively, and for the corresponding malts 94.1 and 67.3 μg per gram. These figures indicate only a small rise in the malted over the unmalted grain and the content of nicotinic acid is considerably greater than the figures reported by Thomas, Bina, and Brown (1942) where only 17 μg per gram was found in barley and 21 μg per gram in the barley malt. The results reported here compare more favorably with the values of 50 μg per gram for whole wheat reported by Melnick, Oser, and Siegel (1941); and 45–70 μg per gram for barley and whole wheat found by Teply, Strong, and Elvehjem (1942).

The average pantothenic acid content of the barley and wheat was found to be 4.5 and 7.9 μg per gram and for the respective malts 5.8 to 9.0 μg per gram. The results represent an increase of low magnitude; however, with every sample the higher results were obtained in the malted grain. The wheat values compare favorably with 8.3 μg per gram of pantothenic acid in whole wheat reported by Strong, Feeney, and Earle (1941) and are slightly low compared to 10–15 μg per gram found by Elvehjem (private communication) and 10 to 11 μg per gram in wheat and barley reported by Jukes (1941).

Discussion and Conclusions

The thiamin, nicotinic acid, and pantothenic acid in the unmalted and malted grains do not show differences of great magnitude, although definite tendencies are evident. From the definite increase in the riboflavin content, the malting process appears to cause a mustering of this food factor for the plant-growth stages that are to follow the period after germination time. It is of interest that none of the B-complex factors except thiamin were consumed or disappeared from the grain to the same extent that dry matter is lost in malting. In general the B-complex factors are apparently conserved to assure proper growth supplements when the reservoirs of grain food have been depleted and other food sources are drawn upon. The thiamin and riboflavin values in wheat and barley compare rather closely, while the nicotinic acid values found were somewhat higher in barley and the pantothenic acid values were higher in wheat.

Summary

Seven samples of barley and five samples of wheat and their respective malts were assayed for their thiamin, riboflavin, nicotinic acid, and pantothenic acid contents. There was a slight decrease in the thiamin value of barley and wheat upon malting. Riboflavin values were found to be three times greater in the malts of both wheat and barley over their respective unmalted grains. Nicotinic acid and pantothenic both showed a slight increase in the malted grain over the unmalted. Barley and wheat respond to malting in much the same manner with respect to the B-complex factors considered in this study. Nicotinic acid values were higher in barley than in the wheat and the pantothenic acid values were higher in wheat.

Acknowledgment

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Literature Cited

Andrews J. S., Boyd, H. M., and Terry, D. E.
1942a The riboflavin content of cereal grains and bread and its distribution in products of wheat milling. Cereal Chem. **19**: 55–64.
1942b Riboflavin analysis of cereals. Ind. Engr. Chem. (Anal. Ed.) **14**: 271–274.
Conner, R. T., and Straub, G. J.
1941a Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem. (Anal. Ed.) **13**: 380–384.
1941b The thiamin and riboflavin contents of wheat and corn. Cereal Chem. **18**: 671–677.
1941c Combined determination of riboflavin and thiamin in food products. Ind. Eng. Chem. (Anal. Ed.) **13**: 385–389.
Downs, D. E., and Cathcart, W. H.
1941 Thiamin content of commercial wheats of the 1940 crop. Cereal Chem. **18**: 796–801.

Hoffman, C., Schweitzer, T. R., and Dalby, G.
1940 The vitamin content of whole-wheat and clear flours. Cereal Chem. **17**: 733–736.
Jukes, T. H.
1941 Distribution of pantothenic acid in certain products of natural origin. J. Nutrition **21**: 193–200.
Laufer, L., Brenner, M. W., and Laufer, S.
1940 Vitamin B_1 levels at various stages of the malting and brewing processes. Proceedings Food Conference, Inst. Food Tech., June 16–19, 1941.
Nordgren, R., and Andrews, J. S.
1941 The thiamin content of cereal grains. Cereal Chem. **18**: 802–811.
Melnick, D., Oser, B. L., and Siegel, L.
1941 The chemical determination of nicotinic acid content of flour and bread. Ind. Eng. Chem. (Anal. Ed.) **13**: 879.
Pennington, D., Snell, E. E., and Williams, R. J.
1940 An assay method for pantothenic acid. J. Biol. Chem. **135**: 213–222.
Schultz, A. S., Atkin, L. and Frey, C. N.
1941 A preliminary survey of the vitamin B_1 content of American cereals. Cereal Chem. **18**: 106–113.
Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin. Ind. Engr. Chem. (Anal. Ed.) **11**: 346.
Snell, E. E., and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**: 675–686.
Strong, F. M., Feeney, R. E., and Earle, Ann
1941 Microbiological assay for pantothenic acid. Ind. Engr. Chem. (Anal. Ed.) **13**: 566–570.
Teply, J., Strong, F. M., and Elvehjem, C. A.
1942 The distribution of nicotinic acid in foods. J. Nutrition **23**: 417–423.
Thomas, J. M., Bina, A. F., and Brown, E. B.
1942 The nicotinic acid content of cereal products. Cereal Chem. **19**: 173–180.

THE CHEMICAL COMPOSITION OF COMMERCIAL HYBRID AND OPEN-POLLINATED VARIETIES OF DENT CORN AND ITS RELATION TO SOIL, SEASON, AND DEGREE OF MATURITY (A PRELIMINARY REPORT) [1, 2]

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Corn is one of the most important crops grown in the United States and it is therefore important to have detailed information about its chemical composition. Not only should its average composition be known, but the effect of variety and environmental factors affecting growth should be studied and understood. This information would be of value to livestock feeders and industrial users of corn. The relatively recent development and widespread use of hybrid corn makes

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such information especially desirable, and very few studies of this nature have been carried out with hybrid corn.

Wimer (1937) studied the composition of mature corn stover as affected by variety, soil type, and fertilizer treatment. He was particularly interested in the effect on the nitrogen, phosphorus, potassium, and calcium content of corn stover from the standpoint of the fertilizer value. The results showed that variety, soil type, and fertilizer treatment were all factors in determining the composition of corn stover.

Neal and Bohstedt (1939) found very little difference in the composition of silage from open-pollinated and hybrid corn.

Morrison (1936) reported the average analysis of well-dried dent corn grain. He stated that the development of high-yielding varieties of corn apparently has brought about, on the average, an appreciable lowering of the fat content, a slight decrease in the protein content, and slight increases in starch and fiber.

Arbuckle and Thies (1926) reported that immature corn is low in protein. Earlier work by these investigators (1924, 1925) led to the conclusion that climate, fertilizer treatment, and variety affect the protein content of corn grain but that soil type affect it very little.

Evans (1941) found that as corn matured, the percentages of protein, crude fiber, sugars, and ash, on the dry-weight basis, decreased in the corn grain for 43 days after pollination, then remained constant. The percentages of starch and ether extract increased steadily to 36 days after pollination, and then remained constant.

Jones and Huston (1914) found that the total amount of crude fiber, fat, ash, crude protein, and starch in the corn plant increased as the plant grew and matured. Their data did not show how these constituents varied in the grain alone.

The work reported in this paper shows the effect of variety, soil type, location, season, and degree of maturity on the chemical composition of corn grain. The results indicate that external growth factors affect the composition of corn grain greatly. Corn grain from different varieties may vary in composition, but there is apparently no consistent correlation between variety and chemical composition.

Materials and Methods

Samples were obtained from replicated corn plots in different locations on different soil types throughout the state of Indiana. Samples of open-pollinated and hybrid varieties of both white and yellow dent corn were studied. For the study of the effect of maturity on the composition of the corn grain, three hybrids on one soil type in one location were used. Table I gives information regarding the samples used for study in each of the three years of the experiment. It should

TABLE I

CONDITIONS UNDER WHICH CORN WAS GROWN DURING THREE SEASONS AND NUMBER OF HYBRIDS AND OPEN-POLLINATED VARIETIES USED EACH YEAR

	Year		
	1938	1939	1940
Total samples	237	259	263
Varieties used			
Total number	43	44	31
Yellow hybrids	27	28	20
Yellow open pollinated	5	5	4
White hybrids	7	7	4
White open pollinated	4	4	3
Number locations	7	8	7
Number soil types	10	11	11

be recognized that not all varieties were grown in every location and on each soil type in any one year. However, 10 varieties were grown in five locations on eight different soil types all three years.

For this work representative samples of ear corn were harvested and artificially dried in a drier with air circulating at a temperature of about 140°F to a moisture content below 10%. The corn was then shelled and stored until chemical analyses could be carried out. The camples of shelled corn were ground to pass a 1-millimeter sieve and the ground samples used for analysis.

Moisture, protein (Kjeldahl-Gunning-Arnold method, copper sulfate as catalyst), ash, crude fat, and crude fiber were all determined by official A. O. A. C. methods (1940). Starch was determined by the method described by Sullivan (1935).

Results and Discussion

The effect of maturity on the percentages of protein, fat, crude fiber and ash in corn grain from hybrids grown in 1938, 1939, and 1940 is shown in Figure 1. The data shown here represent an average analysis of three different hybrids grown at Lafayette on Brookston silt loam. Harvests were made weekly beginning 21 to 30 days after mid-silking date.

As the corn matured, the percentages of protein, fiber, and ash decreased and then remained constant. The percentage of fat increased and then remained constant or decreased slightly. In general the same changes occurred as the corn matured in all three years. It should be noted that the protein content was high at all stages of maturity in 1940 and that the percentage of fat was low at all stages of maturity in 1938. This indicates that seasonal variations may be quite important in determining the composition of corn grain.

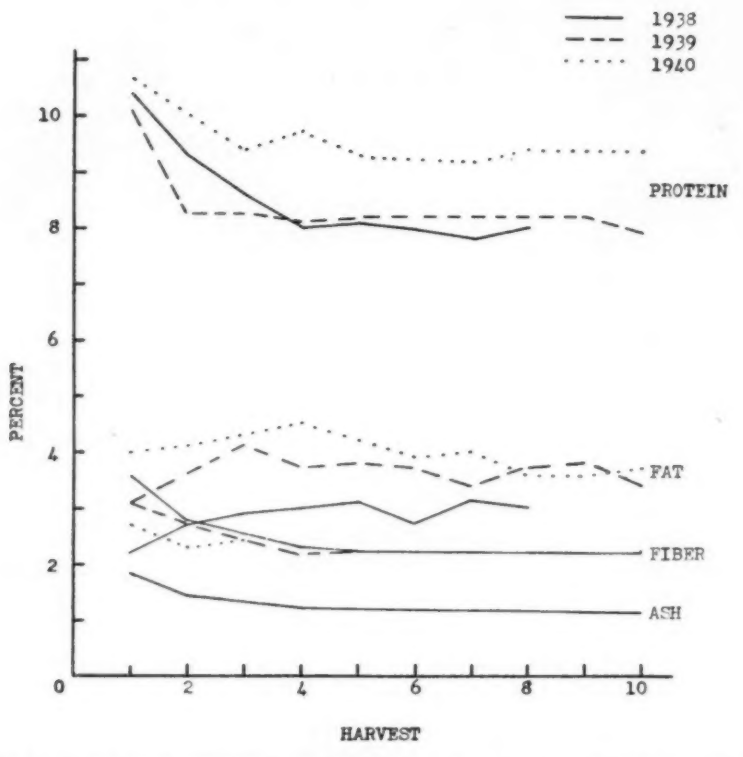

Fig. 1. The effect of maturity on the composition of corn grain from hybrids grown in 1938, 1939, and 1940 as shown by weekly harvests. Percentages are expressed on a 15% moisture basis.

Figure 2 shows the effect of maturity on the composition of grain from the three hybrids grown in 1939 at Lafayette on Brookston silt loam. There were no significant differences in the percentages of fat, fiber, and ash in the three hybrids except at the early harvest periods. Grain from Ind. hybrid 829 contained slightly more protein at all stages of maturity than did grain from the other hybrids. Although the data are not reported, similar results were obtained in 1938 and 1940. That is, the composition of grain from all three hybrids changed in about the same way as the corn matured. There were no significant differences in composition of the grain from the hybrids in any one year.

The data in Table II show the effect of soil type, location, and season on the composition of corn grain. Thirteen yellow varieties were grown in all three years in the locations shown in the table. The average composition of the grain from the different varieties and the range in composition among the varieties is given. Although detailed data are not given, it should be pointed out here that there were no significant differences in composition among the varieties. That is, no

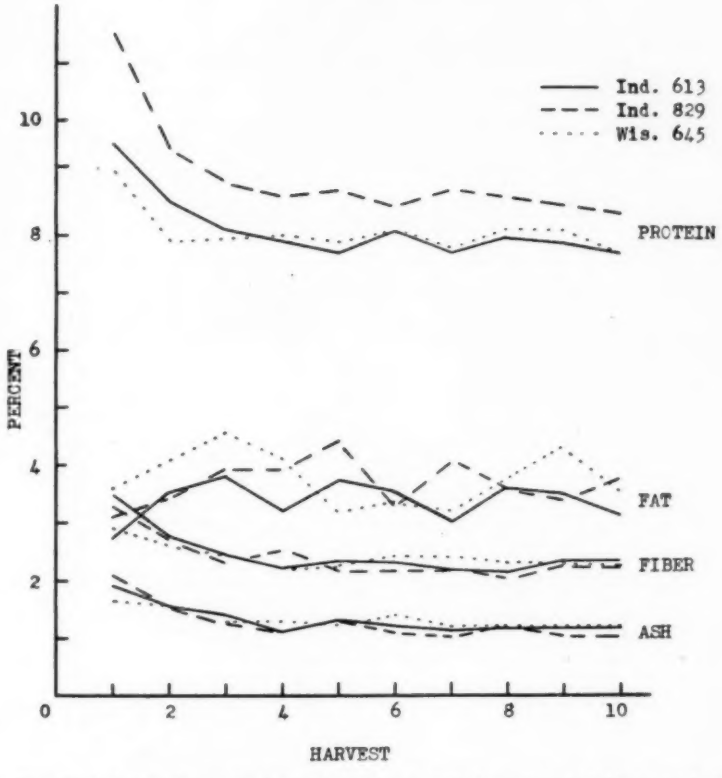

Fig. 2. The effect of maturity on the composition of corn grain from three hybrids grown in 1939 as shown by weekly harvests. Percentages are expressed on a 15% moisture basis.

variety seemed to be consistently different in composition from any other variety.

The effect of season, location and soil type can be seen quite readily. The grain from corn grown in 1938 contained significantly lower percentages of protein and fat than that from corn grown in 1939 and 1940. The protein content of the grain was affected by soil type and location as well as by seasonal differences. However, it should be noted that the effect of soil type and location was not consistent from season to season. For example, the corn grown at Wanatah on muck soil in 1938 contained higher percentages of protein than that grown on Plainfield fine sandy loam, but in 1939 and 1940 the reverse was true. Similarly the corn grown in 1938 at Lafayette on Clyde silty clay loam contained more protein than that grown on Brookston silt loam, but the reverse was true in 1939 and 1940. Thus it appears that it would be impossible to predict the differences in protein content of corn grain grown on different soil types in different locations in different seasons.

TABLE II

EFFECTS OF SOIL TYPE, LOCATION, AND SEASON ON COMPOSITION OF CORN GRAIN—RESULTS EXPRESSED ON 15% MOISTURE BASIS

	Protein (%)			Fat (%)			Ash (%)		
	1938	1939	1940	1938	1939	1940	1938	1939	1940
WANATAH PINNEY PURDUE FARM									
Maumee loam [1]	7.4–8.5	8.3–9.2	8.1–9.4	2.7–3.3	2.7–4.1	3.3–3.9	1.0–1.2	1.0–1.2	0.9–1.1
	Av 7.9	8.8	8.9	2.9	3.5	3.6	1.11	1.07	.99
Muck	7.9–9.3	8.1–9.0	10.1–10.8	2.3–3.3	2.8–3.8	3.1–4.1	1.0–1.2	0.1–1.1	1.0–1.4
	Av 8.7	8.6	10.3	2.8	3.5	3.5	1.11	1.03	1.14
Plainfield fine sandy loam	6.6–7.7	9.1–10.2	10.1–11.8	2.4–3.3	2.8–4.1	3.4–4.0	1.0–1.4	1.2–1.4	1.2–1.5
	Av 7.1	9.9	10.7	2.8	3.5	3.7	1.20	1.28	1.17
TALBOT									
Dark prairie soil	7.7–8.6	8.5–9.9	9.1–10.7	2.4–3.1	2.9–4.1	3.1–4.0	1.0–1.2	1.1–1.4	0.8–1.0
	Av 8.1	9.4	10.0	2.8	3.7	3.6	1.14	1.24	.91
LAFAYETTE SOILS AND CROPS FARM									
Clyde silty clay loam	7.3–8.3	7.5–8.5	7.2–8.9	2.5–3.5	3.0–4.1	2.9–3.9	1.1–1.4	1.1–1.2	1.1–1.5
	Av 7.7	7.9	7.9	3.0	3.1	3.6	1.23	1.16	1.23
Crosby silt loam	7.0–7.8	8.3–9.8	8.3–9.6	2.2–3.2	2.8–3.8	3.3–4.2	1.2–1.3	1.0–1.2	1.0 1.3
	Av 7.3	8.9	9.0	2.9	3.4	3.9	1.24	1.08	1.20
Brookston silt loam	6.2–7.8	8.0–9.1	8.6–10.5	2.3–3.4	2.7–3.9	3.5–4.1	1.0–1.2	1.2–1.4	1.1–1.3
	Av 6.7	8.4	9.1	2.9	3.5	3.8	1.15	1.26	1.20
FARMLAND H. D. FORESTRY FARM									
Clyde silty clay loam	7.5–8.7	8.5–9.8	8.7–10.3	2.1–3.2	3.0–4.6	3.5–4.1	1.0–1.2	1.1–1.4	1.0–1.2
	Av 8.2	9.2	9.5	2.6	3.9	3.8	1.16	1.25	1.05
Crosby silty clay loam	7.9–9.3	8.8–9.8	8.8–10.8	2.0–2.7	3.4–4.1	3.2–4.0	1.0–1.3	1.1–1.2	1.0–1.2
	Av 8.5	9.3	9.4	2.3	3.7	3.6	1.12	1.16	1.07
MILROY R. K. FARM									
"Black and clay" soil	6.6–8.0	7.2–8.3	6.9–7.7	2.3–3.1	2.4–4.2	3.4–4.3	1.1–1.4	1.1–1.4	1.0–1.3
	Av 7.2	7.9	7.3	2.7	3.6	3.9	1.23	1.22	1.12

[1] The range in percentage composition is shown above and the average below.

The starch content of corn grain from 13 varieties grown in 1938 is given in Table III. Although there was considerable variation of the samples, there was no correlation between starch content and variety, soil type, or location. Starch determinations were not made on the samples obtained in 1939 and 1940.

A comparison of the composition of white and yellow varieties of dent corn is shown in Figure 3. The percentages shown are averages from several varieties in each case. It can be readily seen that there was very little difference in the average composition of the white and yellow varieties in any one year when they were grown in the same location. The effect of season and soil type and location on the protein content of the grain from both white and yellow varieties is apparent.

TABLE III

PERCENTAGE OF STARCH IN CORN GRAIN—1938 SAMPLES—RESULTS EXPRESSED ON A 15% MOISTURE BASIS

	Wanatah, Indiana			Lafayette, Indiana			Farmland, Indiana	
	Maumee loam	Muck	Plainfield fine sandy loam	Clyde silty clay loam	Crosby silt loam	Brookston silt loam	Clyde silty clay loam	Crosby silty clay loam
Ind. 417	54.9	54.6	54.1	51.2	55.1	52.4–57.6	54.4	53.3
Ind. 425	51.2	49.8	54.9	56.7	52.7	56.8	52.0	55.0
Ind. 608B	54.0	55.5	52.1	54.7	56.7	53.9	52.6	53.7
Ind. 610	56.2	55.0	55.9	55.5	54.2	54.1	52.8	53.9
Ind. 613	55.3	56.6	53.2	54.3–56.1	53.1–56.3	54.4–55.2	52.2	55.6
Ind. 632D	56.3	53.6	56.0	54.0	53.3	52.5	56.3	53.7
Ind. 829	53.0	54.5	55.2	49.8	55.8	55.6	53.5	52.2
Ind. 842	51.0	55.3	53.7	54.5	55.5	56.1	52.4	51.3
Ind. 844	55.0	52.6	55.4	54.4	51.2	53.7	54.7	54.3
Ind. 845	57.3	50.8	55.1	54.1	54.6	54.9	56.0	52.8
Krug	54.9	55.1	51.5	58.6	52.8	51.2	52.3	56.3
Bryant Reid	51.9	53.1	55.2	53.0	52.7	52.2	53.8	54.5
Purdue 11 Reid			48.6	52.5	51.6	53.2	52.4	52.0
Av	54.2	53.9	53.9	54.2	53.9	54.2	53.5	53.7
Range	51.0–57.3	50.8–56.6	48.6–56.0	51.2–58.6	51.2–56.7	51.2–56.8	52.0–56.3	51.3–56.3

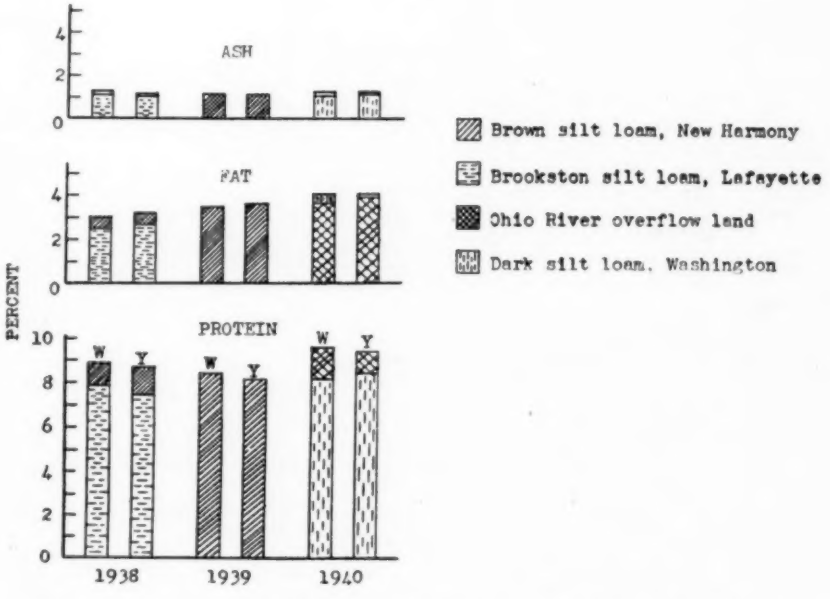

Fig. 3. The composition of grain from white and yellow varieties of dent corn grown in different years on different soil types in different locations. Percentages are expressed on a 15% moisture basis.

Although the work reported here does not show any consistent relationship between variety and chemical composition, one must not conclude that varieties of consistently different chemical composition cannot be developed. In fact, it is quite generally recognized that such varieties can be developed. However, the work does show that

many of the commercial hybrids that have been developed, and the selections of open-pollinated varieties that have been made, produce corn grain of very similar chemical composition.

Summary

As corn matured, the percentages of protein, fiber, and ash in the grain decreased and then remained constant. The percentage of fat increased and then remained constant or decreased slightly. These changes occurred in three hybrids in all three seasons.

Seasonal variations were responsible for variations in the protein and fat content of corn grain. The protein content is also affected by soil type and location.

There were no significant differences in the chemical composition of more than 40 commercial hybrids and open-pollinated varieties of dent corn. The composition of yellow varieties tested was practically the same as that of white varieties grown under the same conditions in the field.

Literature Cited

Association of Official Agricultural Chemists
1940 Methods of analysis (5th ed.), Association of Official Agricultural Chemists, Washington, D. C.

Arbuckle, H. B., and Thies, O. J., Jr.
1924 Variation of protein content of corn, III. J. Elisha Mitchell, Sci. Soc. **40**: 176–180. Chem. Abs. **19**: 2970.
1925 Variation of protein content of corn, IV. J. Elisha Mitchell, Sci. Soc. **41**: 64–69. Chem. Abs. **20**: 62.
1926 Variation of protein content of corn, V. J. Elisha Mitchell, Sci. Soc. **42**: 113–117. Chem. Abs. **21**: 3649.

Evans, J. W.
1941 Changes in the biochemical composition of the corn kernel during development. Cereal Chem. **18**: 468–473.

Jones, W. J., Jr., and Huston, H. A.
1914 Composition of maize at various stages of its growth. Purdue University Agr. Exp. Sta. Bul. 175.

Morrison, F. B.
1936 Feeds and feeding (20th ed.), The Morrison Publishing Co., Ithaca, New York.

Neal, N. P., and Bohstedt, G.
1939 A comparison of the nutritive values of hybrid and open-pollinated corn. Wisconsin Agr. Exp. Sta. Special Bulletin.

Sullivan, J. T.
1935 The estimation of starch in plants with special reference to woody plants. J. Assoc. Offic. Agr. Chem. **18**: 621–636.

Wimer, D. C.
1937 Composition of mature corn stover as affected by variety, soil type, and fertilizer treatment. Ill. Agr. Exp. Sta. Bul. 437.

EFFECT OF CERTAIN VARIABLES IN TECHNIQUE ON THE BREAKING STRENGTH OF LARD PASTRY WAFERS

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Published results of breaking-strength studies with lard wafers have shown a great deal of variation, even though the same fats were under consideration by the different investigators. These variations are indicated in Table I, which shows published breaking-strength figures for lard wafers of 5 to 16 ounces, and for hydrogenated cotton-

TABLE I

BREAKING STRENGTH OF WAFERS AS REPORTED BY DIFFERENT INVESTIGATORS

Fat	Investigator				
	Denton, Gordon and Sperry (1933)	Fisher (1933)	Harvey (1937)	Lowe, Nelson, and Buchanan (1938)	Swartz [1] (1937–41)
	oz	oz	oz	oz	oz
Lard	—	9–16	6–11	5–11	13–44
Hydrogenated cottonseed oil	14–20	13–22	8	9–11	17–37
Vegetable compound	—	13	8	—	19–20
Hydrogenated lard	—	14–21	—	9–12	25–26

[1] Unpublished.

seed oil from 8 to 22 ounces, with similar variations for vegetable compounds and hydrogenated lard. These large variations in results were due at least in part to the conditions chosen for the breaking-strength tests. Each investigator has independently established the conditions judged to be best, without conforming to the formula or technique of predecessors. This is shown in Table II.

The conditions used by Denton, Gordon, and Sperry (1933), by Fisher (1933), and by Harvey (1937), were established by them as standard for all their tests, while the work of Lowe, Nelson, and Buchanan (1938) and of Swartz (1937–41, unpublished) included, along with a standardized technique, additional studies of the influence of some of the conditions on the breaking-strength results.

Lowe reported that, with other factors standardized, the temperature of the fat influenced the breaking strength of the wafers: room-temperature of the fat resulted in more tender wafers than either cold or melted fat. She found also that increased mixing time before adding water made for lower breaking strength, while increased mixing time

TABLE II

CONDITIONS OF BREAKING-STRENGTH TESTS REPORTED IN TABLE I

	Denton, Gordon and Sperry	Fisher	Harvey	Lowe	Swartz
Type of flour	Pastry and family	Pastry	?	All-purpose	Pastry
Percent fat	47	41–44	50	40	44
Percent water	27	25	31	25	25–40
Temp. of flour, °F	46–48	Room	?	75–77	35–97
Temp. of fat, °F	46–48	45–50	?	41–140	35–97
Temp. of water, °F	46–48	45–50	45	24–25	35–97
Mixing time before adding water, min	5	2	2	2½–3½	2
Mixing time after adding water, sec	60	45	45	15–45	30–90
Thickness of wafers, in	⅛	⅛	⅛	3/32	⅛

after adding the water gave greater breaking strength to pastry wafers made with lard.

In view of the absence of an accepted standardized test for breaking strength of pastry wafers, the study to be reported here was undertaken in order to give a clearer picture of the effect of some of the variables that have existed in the formulas and mixing procedures used by the different investigators. Specifically, the factors studied were amount of water used, temperature of ingredients, and length of mixing time after water was added.

Experimental Work

Pastry wafers were made from commercially steam-rendered lard according to the following formula and procedure:

Constants		*Variable*
Pastry flour	200 g	Water, tap—50, 65, and 80 ml
Fat	88	
Salt	5	

Temperature of Ingredients

Flour and salt—room temperature, 67–81°F, usually 73–76°F
Fat and water—40–45°F and 70–75°F

Mixing Time

Before adding water—2 minutes
After adding water—30, 45, 60, or 90 seconds

Mixing Procedure

Flour and salt were sifted together, the fat was added, and mixed in with the pastry knife of a Kitchen-Aid machine (3-qt bowl) on low speed for 60 seconds (70 revolutions). The machine was stopped, the sides of the bowl scraped down with a few turns of the pastry knife, and the mixing continued for an additional 60 seconds. The water was added in less than 5 seconds while the mixer was running, and the mixing was continued for the remainder of the time indicated, 30 to 90 seconds. The dough was formed into a ball with 6–10 motions of the hands. It was rolled out on a floured canvas pastry cloth with a rolling pin covered with a floured stockinette, first to a thickness of 5/16 inch in ten motions, with guide strips to control

thickness. Then the dough was turned at right angles to its former position and rolled to ⅛-inch thickness in 44 further rolling motions. The dough was then cut with a pastry wheel into wafers, each 1½ by 2½ inches, lengthwise of the dough. Each wafer was pricked with a tool containing 15 blunt points to prevent blistering and placed on an aluminum baking sheet. Each baking consisted of 27 wafers. The wafers were chilled for about 10 minutes at 40°–45°F prior to baking.

Baking, Cooling, and Testing Procedure

The wafers were baked 18 minutes in a gas oven at 425°F, and cooled on a wire rack at room temperature for 45 minutes before breaking. The two most atypical wafers (from the standpoint of color or texture) were discarded and the remaining 25 were broken with a Bailey Shortometer.

Number of Wafers Tested

Four bakings totaling 100 wafers were made with each combination of variables.

Results

The results of the breaking strength tests are shown in Table III and Figures 1 and 2. It can be seen that with increasing water the

TABLE III

BREAKING-STRENGTH OF PASTRY WAFERS MADE WITH LARD

(Each figure represents the average of 100 wafers from 4 bakings)

Temperature of fat and water	Water	Blending time after adding water	Breaking strength		Standard deviation	Probable error	Estimated standard error of mean
			Mean	Range			
	%	*sec*	*oz*	*oz*			
40°–45°F	25	30	15.1	5–35	4.7	3.2	0.47
		45	16.7	8–30	4.4	2.9	0.44
		60	15.8	9–27	3.3	2.2	0.33
		90	19.9	12–27	3.2	2.1	0.32
	32.5	30	23.0	9–36	5.7	3.8	0.57
		45	25.9	11–44	5.5	3.7	0.55
		60	29.8	19–45	5.9	4.0	0.59
		90	31.4	22–43	4.6	3.1	0.46
	40	30	33.5	14–53	8.3	5.6	0.83
		45	37.2	20–64	7.1	4.8	0.71
		60	43.7	29–56	5.9	4.0	0.59
		90	42.1	27–54	5.2	3.5	0.52
70°–75°F	25	30	13.9	7–21	2.8	1.9	0.28
		45	14.2	8–30	3.8	2.6	0.38
		60	15.5	10–22	2.6	1.8	0.26
		90	14.4	7–23	3.7	2.5	0.37
	32.5	30	24.1	14–33	4.5	3.0	0.45
		45	22.5	15–31	3.6	2.4	0.36
		60	24.8	14–35	4.3	2.9	0.43
		90	22.8	13–34	3.7	2.5	0.37
	40	30[1]	36.5	25–56	7.2	4.9	0.83
		45	34.8	20–50	5.4	3.7	0.54
		60	34.2	24–46	4.3	2.9	0.43
		90	30.7	18–39	4.0	2.7	0.40

[1] 75 wafers only, instead of 100.

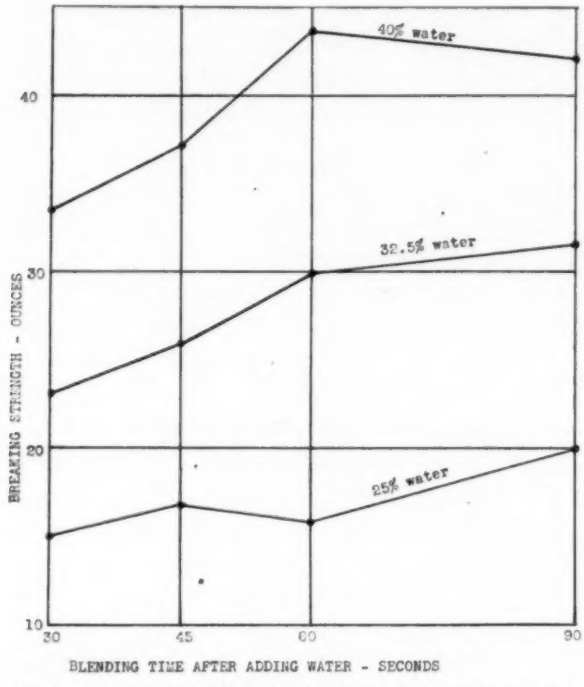

Fig. 1. Breaking strength of pastry wafers—fat and water 40°–45°F.

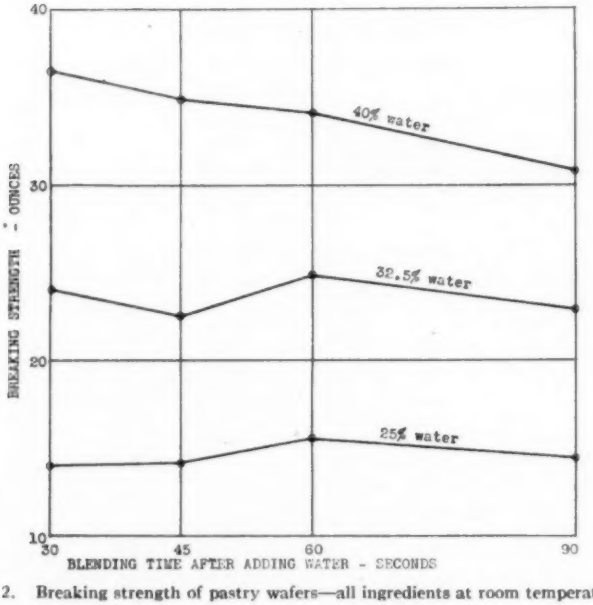

Fig. 2. Breaking strength of pastry wafers—all ingredients at room temperature.

breaking strength increased strikingly, for example from 15.8 ounces with 25% water, to 43.7 ounces with 40% water, at 60 seconds' mixing time, using cold fat and water. Under other conditions of temperature and mixing times the same effect is shown, but to a lesser degree.

As the mixing time was increased, there was little effect on breaking strength, especially when the ingredients were all at room temperature. An exception to this is seen at 90 seconds' mixing, 40% water, where the breaking strength was somewhat lower. When the fat and water were cold, there was some increase in breaking strength with increased mixing time, especially at the highest water content.

Using the fat and water at room temperature instead of at ice-box temperature resulted in lower breaking strengths, at most of the mixing times and levels of water.

Discussion

The results of this study agree with the conclusions reported by Lowe and co-workers and also with their conception of the action of the ingredients of the dough. Specifically, in both this study and in Lowe's, increased mixing time after adding the water made for greater breaking strength in the wafers, especially when the fat and water were cold. This is to be expected, for increased manipulation of the dough brings about greater opportunity for hydration and development of the gluten of the flour. But when the fat and water were at room temperature, the time of mixing seemed to have little effect on the breaking strength of the wafers. This could be a direct result of the greater plasticity of the lard at the higher temperature. The fat, being more plastic, more completely covers the flour, giving it a protective waterproof coating that prevents a part of the subsequent hydration that would otherwise have occurred. This same conception also accounts for the generally lower breaking strength of all the wafers made from ingredients at room temperature, compared to those made from fat and water at ice-box temperature.

The effect of increasing the amount of water can also be explained on the same basis. Additional water makes possible more nearly complete hydration of the gluten, with consequently higher breaking strength in the resulting wafers.

Summary

The results of this study of breaking strength of pastry wafers can be summarized in the following outline form.

Variable	*Result*
1. Increased water (from 25 to 40%).	1. Progressive increase in breaking strength.
2. Increased mixing time after adding water (from 30 to 90 seconds).	2. (*a*) Fat and water cold: greater breaking strength. (*b*) All ingredients room temperature: same or lower breaking strength.
3. Room temperature for ingredients instead of ice box temperature.	3. Decrease in breaking strength.

Literature Cited

Denton, M. C., Gordon, B., and Sperry, R.
1933 Study of tenderness in pastries made from flours of varying strengths. Cereal Chem. **10**: 156–160.
Fisher, J. D.
1933 Shortening value of plastic fats. Ind. Eng. Chem. **25**: 1171–1173.
Harvey, A. W.
1937 Shortening properties of plastic fats. Ind. Eng. Chem. **29**: 1155–1160.
Lowe, B., Nelson, P. M., and Buchanan, J. H.
1938 The physical and chemical characteristics of lards and other fats in relation to their culinary value. I. Shortening value in pastry and cookies. Iowa Agr. Exp. Sta. Res. Bul. 242.

INACTIVATION OF ALPHA- AND BETA-AMYLASE IN AQUEOUS MALT EXTRACTS [1]

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The constitution of the amylolytic enzyme system in malt has been studied extensively with regard to the separation, characterization, and accurate determination of the components. In the hydrolysis of a raw starch paste by malt extract, three general types of reaction are recognized: (1) liquefaction (accompanied by a liberation of esterified phosphorus), (2) dextrinization, or progressive diminution of the iodine-coloring capacity of the starch, and (3) saccharification, apparent notably as a production of maltose. Enzymes catalyzing each of the three types of reactions mentioned above have been described, and their partial or complete isolation reported.

Thus, according to Waldschmidt-Leitz and Mayer (1935), amylophosphatase catalyzes the liquefaction and concomitant liberation of esterified phosphorus from the starch paste; the dextrinogenic- or alpha-amylase is active in the dextrinization of the starch to low-molecular-weight, noniodine-staining dextrins. The rapid production of maltose is ascribed mainly to the action of the saccharogenic- or beta-amylase,

[1] Cooperative investigations between the University of Wisconsin and the U. S. Department of Agriculture, Division of Cereal Crops and Diseases, Bureau of Plant Industry. The United States Maltsters Association and the Malt Research Institute have cooperated through Industrial Fellowship grants to the University of Wisconsin.

although a comparatively small amount of maltose is formed by alpha-amylase as well.

The view that alpha-amylase is active in the liquefaction as well as in the dextrinization of a starch substrate has been advanced by Blom, Bak, and Braae (1937), Blom and Bak (1938), and Józsa and Johnston (1935). The parallelism between the two actions has recently been studied by Hollenbeck and Blish (1941) under several conditions. They concluded on the basis of their results that both reactions were due to the same enzyme. On the other hand, Mayer (1939) showed that raw starch paste oxidized by a minimal amount of iodine did not result in the production of reducing materials by malt extract, but could still be liquefied.

Several techniques have been developed for the partial or complete separation of the amylases present in malt extract. The methods of Waldschmidt-Leitz, Reichel, and Purr (1932) for the selective adsorption of beta-amylase on alumina at pH 3.8, or the adsorption of alpha-amylase on mature starch grains (Holmbergh, 1933) are well known. Other methods for the separation of the two amylase components have been developed by Ohlsson (1926, 1930) and Blom, Bak, and Braae (1937). The Ohlsson technique makes possible the destruction of beta-amylase in the presence of alpha-amylase by heating the neutral green malt extract at 70° for 15 minutes. Several workers, including Ohlsson (1926), have found that at least 15–16% of the alpha-amylase is destroyed by this treatment. Ohlsson (1926) also found that alpha-amylase was almost quantitatively destroyed by treatment of an acidified extract at pH 3.3 and 0° for 15 minutes, the destruction of beta-amylase being negligible. Blom, Bak, and Braae (1937) used less drastic conditions for inactivation by heat and by low pH and low temperature. They found that acidification of a malt amylase preparation at 20° and pH 4.2 resulted in almost complete destruction of alpha-amylase in four hours, and heating the neutral enzyme suspension at 60° for one hour resulted in a constant ratio of reducing power/liquefaction, which they took as an indication of a one-component (alpha-amylase) system.

In our work a study was made of the inactivation of beta-amylase at 60°, 65° and 70°C, and of alpha-amylase at pH 3.3 and 0°C in aqueous extracts of malts exhibiting rather wide differences in the activity of the amylase system originally present. The principal object in this study was an examination of the rates of the inactivations. Improved and specific methods for alpha-amylase and the determination of total saccharifying activity were applied to the partially inactivated extracts.

Experimental Material

Three malts were chosen for study, exhibiting high, low, and intermediate diastatic activities. The high diastatic malt, Peatland, had an activity of 752 mg maltose (188°L), the low, Atlas, 288 mg maltose (72°L), and the intermediate, Oderbrucker, 448 mg maltose (112°L).

Heat Inactivation

Twenty-five-gram portions of ground malt were extracted with 500 ml of water in a 20° bath for two hours, and filtered for one-half hour. Twenty-five-ml aliquots of the extract were transferred to a series of 8-inch test tubes, and placed in a bath adjusted to the desired inactivation temperature. The temperature of the bath was maintained manually to within $\pm 0.5°$ of the desired temperature. Tubes were removed from the bath at appropriate time intervals and transferred immediately to a water bath at 0–4°C, and cooled to 10–15°C. The cooled, partially inactivated extracts were transferred to 100-ml volumetric flasks, made to the mark with water and mixed thoroughly. These diluted solutions were used in all subsequent determinations.

Low-Temperature, Low-pH Inactivation

The same method for preparation of the malt extract was used here as was used for the heat inactivations. Forty-ml portions of the extract were measured into 50-ml volumetric flasks and placed in an ice-water bath (0°). When the contents of each flask had reached 0°, the acidity was adjusted to pH 3.3 with HCl (0.025 or 0.05*N*) with vigorous shaking. At appropriate time intervals, sufficient *N*/14 NaOH was added with constant agitation to bring to pH 5.1. The necessary amounts of both acid and base required for pH adjustments were determined on a separate portion. The partially inactivated extracts were allowed to attain room temperature, made to mark with water and used in the subsequent determinations. All runs on both heat- and low-temperature, low-pH inactivations were carried out in duplicate.

Methods

In designing the experimental section of this work, an attempt was made to use methods useful in the determination of one component. It was convenient to use a method specific for alpha-amylase. No attempt, however, was made to differentiate between the saccharifying activity due to alpha-amylase and that due to beta-amylase. In the heat-inactivation treatments, the large decreases in saccharifying activity undoubtedly were due primarily to a loss of beta-amylase activity, and the small decreases in saccharifying activity at 0° and

pH 3.3 were ascribed principally to the effect of alpha-amylase destruction. This conclusion seems justified (see later). The method of Kneen and Sandstedt (1941) was used for determination of total saccharifying activity, using 0.05*N* instead of 0.1*N* potassium ferricyanide.

The dextrinizing activity of the partially inactivated extracts by use of 2% soluble starch as substrate (Wohlgemuth, 1908) has been shown to be affected seriously by variations in beta-amylase content (Hanes and Cattle, 1938). However, it was used in this work to compare with the other dextrinization procedure.

The improved Wohlgemuth procedure, developed by Sandstedt, Kneen and Blish (1939), is designed to circumvent this complication of variations in beta-amylase by use of standard alpha-amylodextrin instead of soluble starch as substrate. In the preparation of the alpha-amylodextrin substrate, these workers recommended the use of hard wheat extract as a suitable source of pure beta-amylase. We have found it convenient to use instead a preparation of soybean amylase. Newton and Naylor (1939) report that soybeans contain very little, if any, dextrinizing activity. For ease in comparison, the same method was used in the soluble starch dextrinization, except of course that the pretreatment with beta-amylase was omitted.

The method of Blom and Bak (1938) was used with minor modifications in the determination of liquefying activity. In unpublished work with this method, we have found that the effect of beta-amylase is of only minor importance in the liquefaction of starch paste, and especially so in concentrations met with in this work. However, attention is called to the values for liquefying activity at the later stages of low-temperature, low-pH inactivation. In these cases, relatively concentrated extracts were used in the determination, and the large excess of beta-amylase may have affected the Blom-Bak values.

Presentation and Discussion of Data

The effect of the various treatments on the extracts of the three varieties seemed to show quantitative differences only. Therefore, in order to conserve space, a plot of the complete set of values for one variety only, Oderbrucker, will be given. The data for all three varieties are given in tabular form. Each figure represents the average of duplicate runs under identical conditions.

Saccharifying Activity

As indicated earlier, the heating of a malt extract at 60°–70° has an adverse affect on the saccharifying activity. The effect of heating an unbuffered extract at 60°, 65°, and 70° on the saccharifying activity is given in graphic and tabular form in Figure 1 and Table I.

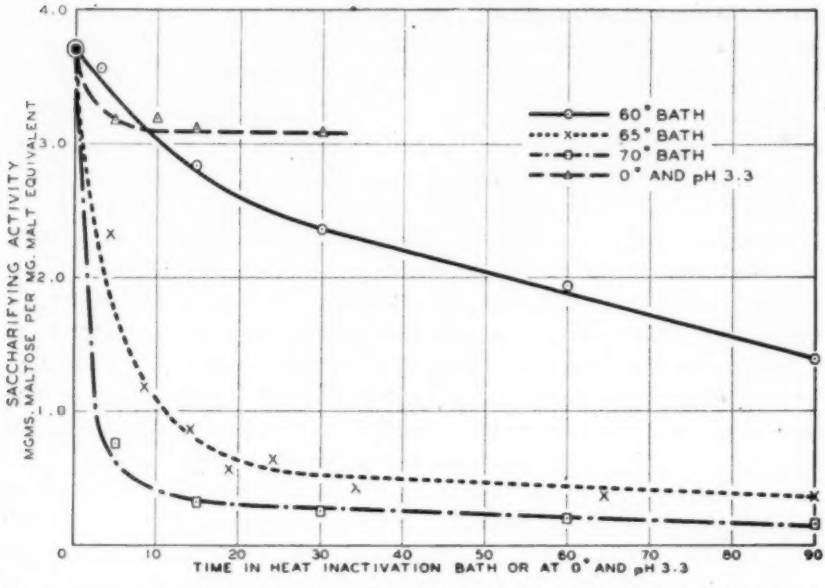


Fig. 1. The rate of inactivation of saccharifying activity of a malt extract (Oderbrucker) as mg maltose/mg malt equivalent vs time (min) treatment at 60°, 65°, 70°C or at 0°C and pH 3.3.

TABLE I

EFFECT OF INACTIVATION TREATMENT ON SACCHARIFYING ACTIVITY

(Mg maltose per mg malt equivalent)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Saccharifying activity—0°C and pH 3.3
ATLAS					
Original	2.34	2.34	2.34	Original	2.34
at $(t-1)°$	2.21	1.62	0.31	5	1.98
15	1.75	0.40	0.26	10	1.95
30	1.34	0.33	0.21	15	1.99
60	0.91	0.27	0.14	30	1.92
90	0.53	0.25	0.10		
ODERBRUCKER					
Original	3.70	3.70	3.70	Original	3.70
at $(t-1)°$	3.56	2.32	0.76	5	3.13
15	2.82	0.60	0.31	10	3.14
30	2.38	0.50	0.26	15	3.10
60	1.94	0.40	0.20	30	3.07
90	1.39	0.30	0.16		
PEATLAND					
Original	6.24	6.24	6.24	Original	6.24
at $(t-1)°$	5.96	4.09	0.61	5	5.70
15	4.98	0.73	0.46	10	5.65
30	4.25	0.58	0.36	15	5.66
60	3.08	0.50	0.23	30	5.65
90	2.55	0.42	0.16		

The loss of saccharifying activity at 60° appears as a continuous function of time, showing little tendency to level out even after 90 minutes. The times required to reach one-half the original activity lie between 40 and 60 minutes for the three varieties. Even though treatment of the malt extract at 60° results in the destruction of the saccharogenic property of malt amylase, the reaction (inactivation) is slow and incomplete within the 90-minute period.

Treatment of the extracts at 65° or 70° is in direct contrast to this. Progressive heating of the malt extracts at 65° or 70° resulted in an extremely rapid and extensive drop in saccharifying activity, which leveled out abruptly to a straight-line and very gradual decline in activity. The point at which leveling out occurred corresponded to about 20 minutes in the bath at 65° and about 10 minutes at 70°. The time required to reach one-half original activity was reduced from about 50 minutes at 60° to 5 minutes at 65° and 1.0–1.5 minutes at 70°. It is also significant that 70% or more of the original activity had been lost before the extracts had reached 69°, and more than 30% before they had attained 64°.

Acidification of the malt extracts to pH 3.3 at 0° resulted in a very rapid though small initial decrease in saccharifying activity, followed by essentially no further change up to the maximum time studied, 30 minutes. It can be seen that by far the greatest fall in activity occurred in less than 5 minutes under these conditions.

Soluble Starch Dextrinizing Activity

The dextrinizing activity of malt extracts is attributed principally to the action of alpha-amylase, but, when acting on 2% soluble starch, the contribution of beta-amylase is significant. Accordingly, the values of the dextrinizing activity of the treated extracts are given in Table II and Figure 2.

By examination of the data for dextrinization of 2% soluble starch as affected by treatment of the extracts at 60°, 65°, and 70°, and also at 0° and pH 3.3, the effect of beta-amylase is readily apparent. However, there does not seem to be any quantitative parallelism between the decrease in saccharifying activity and decrease in dextrinizing activity on soluble starch. Certainly, from these findings, one is forced to agree with Hanes and Cattle (1938) that the part played by beta-amylase in the dextrinization of soluble starch is not understood. By comparison of the curves for soluble starch-dextrinizing activity of the heat-treated extracts with the corresponding curves for saccharifying activity, a rather general qualitative similarity shows up. Thus, soluble-starch dextrinizing activity decreases as a curvilinear, continuous function of time at 60°. The large initial decrease in

TABLE II

EFFECT OF INACTIVATION TREATMENT ON SOLUBLE STARCH DEXTRINIZING ACTIVITY

(Sandstedt-Kneen-Blish units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Dextrinizing activity—0°C and pH 3.3
ATLAS					
Original	12.4	12.4	12.4	Original	12.4
at $(t-1)°$	—	10.4	5.0	5	1.0
15	10.2	5.5	4.4	10	0.9
30	8.8	5.0	3.3	15	0.9
60	7.2	4.2	2.1	30	0.8
90	6.3	3.3	1.5		
ODERBRUCKER					
Original	16.6	16.6	16.6	Original	16.6
at $(t-1)°$	—	—	5.9	5	1.5
15	14.7	7.2	5.1	10	1.4
30	13.0	6.0	4.4	15	1.4
60	11.2	5.2	3.2	30	1.3
90	9.3	4.5	2.5		
PEATLAND					
Original	29.4	29.4	29.4	Original	29.4
at $(t-1)°$	—	23.3	9.4	5	2.7
15	24.6	10.6	8.2	10	2.3
30	22.1	9.5	6.0	15	2.3
60	17.5	8.0	3.6	30	2.2
90	16.4	6.7	2.3		

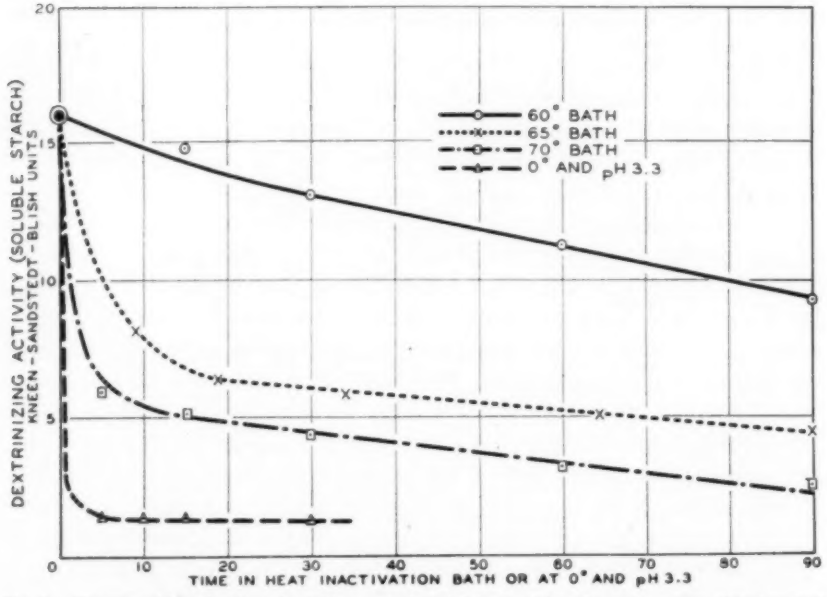


Fig. 2. The rate of inactivation of soluble starch-dextrinizing activity of a malt extract (Oderbrucker) as Sandstedt-Kneen-Blish units vs time (min) treatment at 60°, 65°, 70°C or at 0°C and pH 3.3.

TABLE III

EFFECT OF INACTIVATION TREATMENT ON AMYLODEXTRIN DEXTRINIZING ACTIVITY

(Sandstedt-Kneen-Blish units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Dextrinizing activity—0°C and pH 3.3
ATLAS					
Original	31.2	31.2	31.2	Original	31.2
at $(t-1)°$	—	30.1	22.7	5	1.5
15	30.1	26.0	21.0	10	1.2
30	28.7	23.5	16.6	15	1.0
60	26.9	20.7	11.2	30	0.9
90	25.4	18.0	9.0		
ODERBRUCKER					
Original	33.6	33.6	33.6	Original	33.6
at $(t-1)°$	—	32.0	26.0	5	1.7
15	33.1	31.2	21.9	10	1.5
30	32.7	29.8	20.5	15	1.5
60	31.3	27.2	15.6	30	1.4
90	30.1	24.5	12.9		
PEATLAND					
Original	63.0	60.0	57.7	Original	57.7
at $(t-1)°$	—	58.2	39.3	5	3.1
15	61.5	50.0	34.4	10	2.7
30	60.0	47.0	25.3	15	2.9
60	56.9	40.0	17.0	30	2.7
90	54.5	34.0	11.8		

saccharifying activity followed by an abrupt leveling out with further treatment at 65° or 70° is apparent also in the soluble-starch dextrinizing activity of the partially inactivated extracts. The times required to attain one-half the original activity varied from more than 90 minutes at 60° to about 10 at 65° and 2 at 70°.

Treatment of the malt extracts at 0° and pH 3.3 resulted in an extremely rapid decline in the soluble-starch dextrinizing activity, closely resembling the corresponding loss in starch liquefying and alpha-amylodextrin dextrinizing activities. However, here again the similarity is qualitative only.

Alpha-Amylodextrin Dextrinizing and Liquefying Activity

The parallelism between dextrinizing and liquefying activities has been discussed previously. While no high correlation between soluble-starch dextrinizing activity and starch-liquefying activity could be established, a remarkably close parallelism between the alpha-amylodextrin dextrinizing and corresponding liquefying activities is apparent. The ratio, liquefying activity/amylodextrin dextrinizing activity, is given in Table V. The ratio is not a constant over the whole set of

TABLE IV

EFFECT OF INACTIVATION TREATMENT ON LIQUEFYING ACTIVITY

(Blom-Bak units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Liquefying activity—0°C and pH 3.3
ATLAS					
Original	43.6	43.6	43.6	Original	43.6
at $(t-1)°$	—	41.2	38.2	5	4.6
15	40.0	38.0	35.0	10	3.7
30	38.9	35.3	24.9	15	—
60	37.7	29.5	16.9	30	—
90	36.2	25.5	13.4		
ODERBRUCKER					
Original	49.6	49.6	49.6	Original	49.6
at $(t-1)°$	—	45.0	42.8	5	5.6
15	47.4	42.3	39.0	10	5.5
30	46.6	40.7	33.4	15	5.2
60	44.4	37.2	25.4	30	5.0
90	43.1	31.2	21.1		
PEATLAND					
Original	82.1	82.1	82.1	Original	82.1
at $(t-1)°$	—	75.7	71.5	5	11.1
15	78.1	68.7	62.2	10	9.2
30	77.4	62.8	45.4	15	9.6
60	71.6	54.0	27.9	30	9.3
90	70.1	45.3	18.9		

data, but in general varies both with treatment and variety. The ratio is constant within reasonable limits for a single variety and treatment. We find it extremely difficult to explain this situation. It is due possibly to lack of uniformity in the alpha-amylodextrin substrate, but we have not checked this.

By reference to Figures 3 and 4, it may be seen that a destruction of saccharifying activity is accompanied always by a decrease in the alpha component as deduced from measurements of liquefying and amylodextrin-dextrinizing activities. Obviously, the higher the inactivation temperature used, the more rapid and extensive the destruction of alpha-amylase activity. A closer examination of the time-vs-activity curves for liquefying and amylodextrin-dextrinizing activity reveals that at 60° the destruction of alpha-amylase approximates a straight line from the outset, while at 65° the initial decrease is rapid, followed again by an approximate straight-line decrease on further treatment. At 70°, the decrease in alpha-amylase activity is curvilinear throughout and rather extensive.

The destruction of both liquefying and dextrinizing (alpha-amylodextrin) activities is rapid and very extensive in the early stages of treatment at pH 3.3 and 0°. All but 10%–13% of the original activity

TABLE V

RATIO OF LIQUEFYING/AMYLODEXTRIN ACTIVITIES OF PARTIALLY INACTIVATED MALT EXTRACT

(Blom-Bak units/amylodextrin dextrinizing units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Liq./amylodextrin activity—0°C and pH 3.3
ATLAS					
Original	1.40	1.40	1.40	Original	1.40
at $(t-1)°$	—	1.37	1.68	5	3.07
15	1.33	1.46	1.67	10	3.08
30	1.36	1.50	1.50	15	—
60	1.40	1.43	1.51	30	—
90	1.43	1.42	1.49		
ODERBRUCKER					
Original	1.48	1.48	1.48	Original	1.48
at $(t-1)°$	—	1.41	1.65	5	3.29
15	1.43	1.36	1.78	10	3.67
30	1.43	1.37	1.63	15	3.47
60	1.42	1.37	1.63	30	3.57
90	1.43	1.27	1.64		
PEATLAND					
Original	1.30	1.37	1.42	Original	1.42
at $(t-1)°$	—	1.30	1.82	5	3.58
15	1.27	1.37	1.81	10	3.41
30	1.29	1.34	1.79	15	3.31
60	1.26	1.35	1.64	30	3.44
90	1.29	1.33	1.60		

was lost in less than 5 minutes. In the three malts studied this initial large decrease was followed by little if any further change. A parallelism may be drawn here between the abrupt initial loss of saccharifying activity and the loss of alpha-amylase activity, which strongly suggests that this reduction in saccharifying activity is due preponderantly to a loss of alpha-amylase.

Thus far the discussion has been confined to a consideration of the effects of inactivation treatment upon the enzyme components present in a malt extract. Several interesting points are apparent in the interrelationship between the several factors.

It seems logical to assume, as did Blom, Bak, and Braae (1937), that the ratio of liquefying activity or dextrinizing activity to the corresponding saccharifying values would be constant when one component only was present. These workers indicated that this situation was obtained by heating suspensions of a purified malt amylase preparation at 60° for a minimum time of one hour. They claim these mild conditions are successful because they were dealing with purified preparations. We were unable to attain constant ratios between these factors at any stage in the 60° heat inactivation. On this basis, we feel justi-

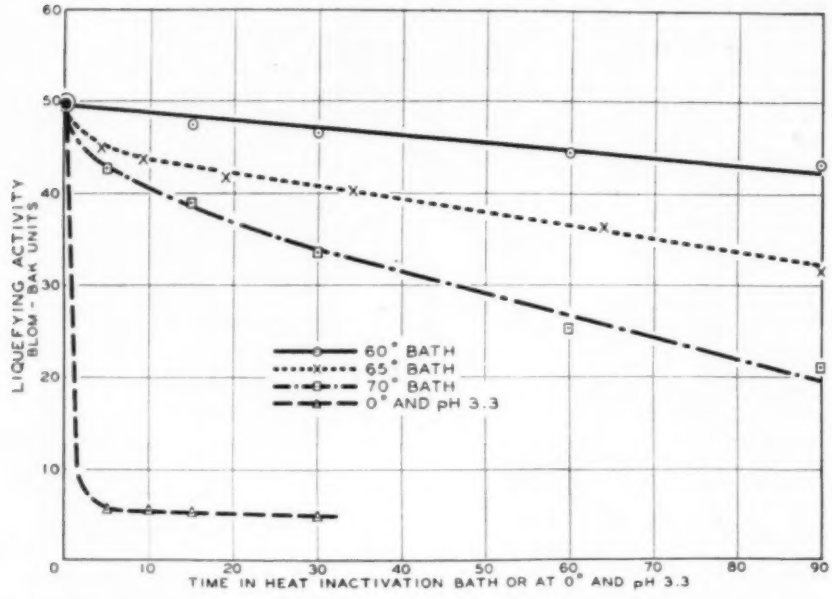


Fig. 3. The rate of inactivation of liquefying activity of a malt extract (Oderbrucker) as Blom-Bak units vs time (min) treatment at 60°, 65°, 70°C or at 0°C and pH 3.3.

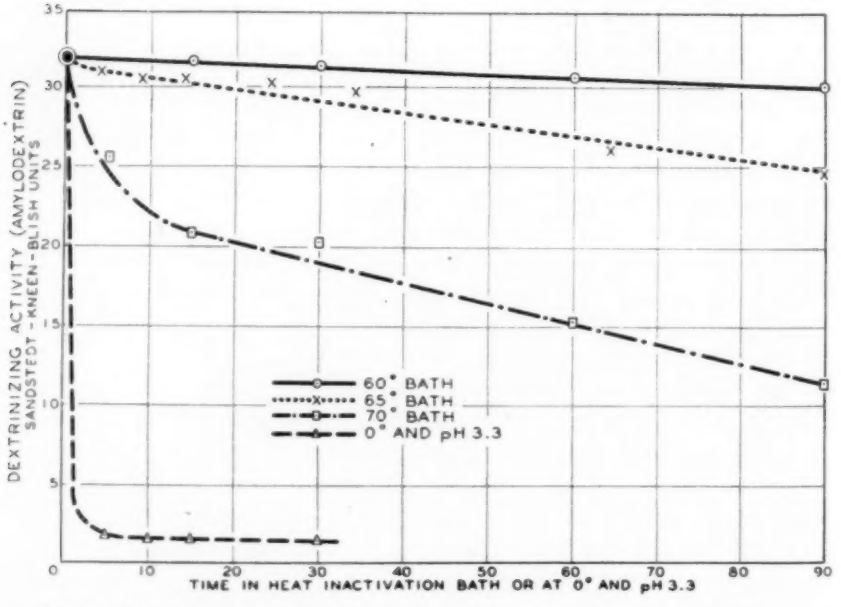

DEXTRINIZING ACTIVITY (AMYLODEXTRIN)
SANDSTEDT - KNEEN - BLISH UNITS
60° BATH
65° BATH
70° BATH
0° AND pH 3.3
TIME IN HEAT INACTIVATION BATH OR AT 0° AND pH 3.3

Fig. 4. The rate of inactivation of alpha-amylodextrin dextrinizing activity of a malt extract (Oderbrucker) as Sandstedt-Kneen-Blish units vs time (min) treatment at 60°, 65°, 70°C or at 0°C and pH 3.3.

fied in concluding that the destruction of beta-amylase in an unpurified malt extract at 60° is incomplete even after 90 minutes. On the other hand, a constant ratio is obtained rather early in the inactivation both at 65° and 70°. A constant ratio at 65° first appears after 15–20 minutes in the bath; the constant ratio at 70° corresponds to approximately 15 minutes, thus confirming the results of Ohlsson (1926).

Summary

The rate and extent of inactivation of the amylase components of malt extracts were measured at 60°, 65°, and 70° and at pH 3.3 and 0°C. Measurements were made of saccharification of soluble starch, liquefaction of raw starch paste, and dextrinization of both soluble starch and amylodextrin on the original and partially inactivated extracts.

Complete inactivation of beta-amylase was not attained at 60° for the maximum time studied, *i.e.*, 90 minutes. At 70° the beta-amylase was completely removed after 15 minutes' treatment, while at 65° approximately 20 minutes were required for complete inactivation. At pH 3.3 and 0° the loss of dextrinizing or alpha-amylase activity was extremely rapid. The maximum effect was obtained usually in 5 minutes. This treatment results in relatively little reduction of saccharifying power, most of this reduction apparently being due to loss of alpha-amylase.

A highly significant correlation between starch-liquefying power and dextrinizing power was found, indicating that these two effects are probably produced by the same component of the diastatic system.

Literature Cited

Blom, J., and Bak, A.
1938 Über die Bestimmung von Amylasen durch Verflüssigung von Stärkekleister. Z. physiol. Chem. **256**: 197–207.
—— and Braae, B.
1937 Alpha-Amylasen-Beta-Amylasen. Z. physiol. Chem. **250**: 104–122.
Hanes, C. S., and Cattle, Margaret
1938 Starch-iodine coloration as an index of differential degradation by the amylases. Proc. Roy. Soc. (London) **B125**: 387–414.
Hollenbeck, C. M., and Blish, M. J.
1941 Parallelism between starch dextrinizing and liquefying activities of amylases. Cereal Chem. **18**: 754–771.
Holmbergh, O.
1933 Über die Adsorption von Alpha-amylase. Bio-chem. Z. **266**: 203–215.
Józsa, S., and Johnston, W. R.
1935 Determination of alpha-amylase. Ind. Eng. Chem. (Anal. Ed.) **7**: 143–146.
Kneen, E., and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18**: 237–252.
Mayer, K.
1939 Über die Stärkeverflüssigung. Z. physiol. Chem. **262**: 29–26.
Newton, J. M., and Naylor, N. M.
1939 Soybean amylase. I. The concentration and characterization of soybean amylase. Cereal Chem. **16**: 71–78.

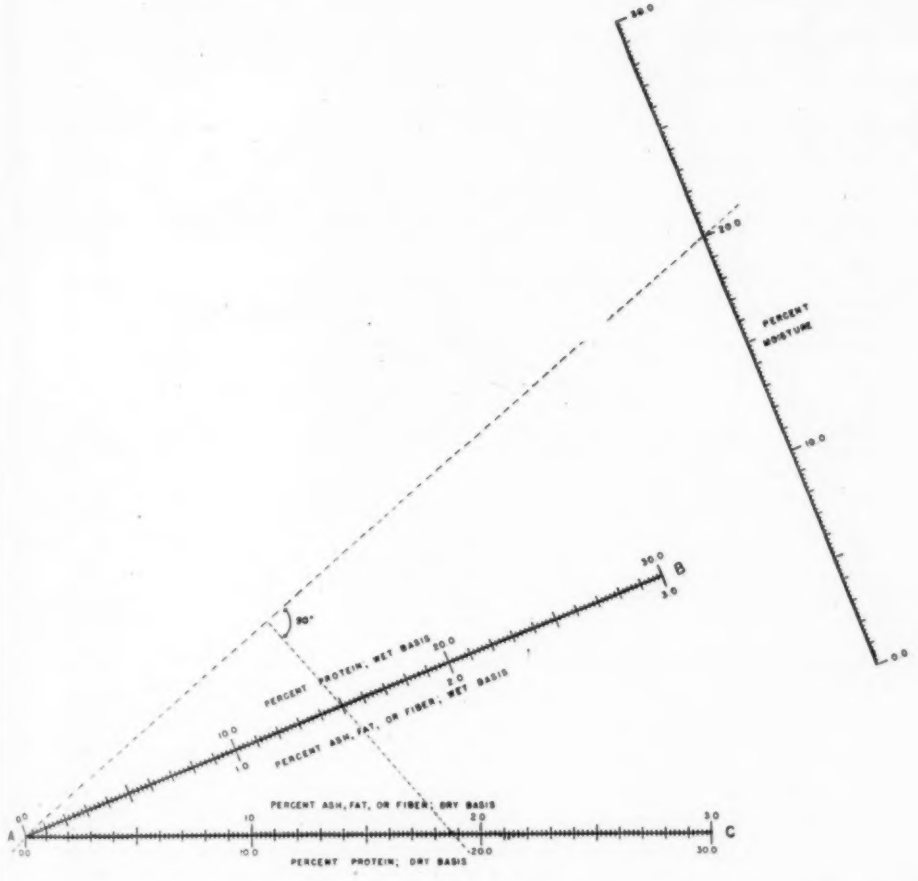
Ohlsson, E.
1926 On the two components of malt diastase. Compt. rend. trav. lab. Carlsberg **16**: 1–68.
1930 Über die beiden Komponenten der Malzdiastase, besonders mit Rücksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. Z. physiol. Chem. **189**: 17–63.
Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.
Waldschmidt-Leitz, E., and Mayer, K.
1935 Enzyme Amylolyse V. Amylophosphatase von Gerste. Z. physiol. Chem. **236**: 168–180.
—— Reichel, M., and Purr, A.
1932 Alpha- and Beta-amylases in malt and barley, Naturwissenschaften **20**: 254.
Wohlgemuth, J.
1908 Über eine neue Methode zur Quantitativen Bestimmung des diastatischen Ferments. Biochem. Z. **9**: 1–9.

A NOMOGRAPH FOR CEREAL LABORATORIES

T. S. LEARY[1] and F. F. FARLEY[2]

(Received for publication July 15, 1942)

A nomograph is presented for converting percentages of protein, ash, fat, and fiber in cereal products to a dry basis. The dotted lines in the illustration shows the use of the nomograph for a cereal product of 20% moisture.



A straight edge is made to coincide with point A and the moisture content (*e.g.*, at 20% moisture). A perpendicular to the straight edge, such as a 90° triangle, is moved along the straight edge until the perpendicular coincides with the known percentage of protein, ash,

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[2] University of Detroit, Detroit, Michigan.

fat or fiber which has been determined on a wet basis (*e.g.*, 15% protein). This value is located on line AB. With the perpendicular in this position the percentage of the desired constituent on a dry basis is read from the nomograph at the point where the perpendicular crosses line *AC* (*e.g.*, 18.7 + percent protein on a dry basis).

In practice a 20-by-20 inch nomograph is used. Values may be read to three significant figures with ease.

BOOK REVIEW

Natural and Synthetic High Polymers. By Kurt H. Meyer, translated by L. E. R. Picken, edited by H. Mark. xviii & 690 pp. Interscience Publishers Inc., 215 Fourth Avenue, New York. Price $11.00.

This is Volume IV in a series of excellent texts on high-polymer chemistry. Together with Volume II it comprises an English edition of *Der Aufbau der hochpolymeren organischen Naturstoffe*.

The beginning chapters are concerned with explanations of the terms polymers, molecular weight, valence, etc. Following this the methods of characterization of high-molecular-weight substances by modern techniques and the interpretation of results are summarized. These techniques include viscosity, electrophoresis, ultracentrifuge, osmotic pressure, and the x-ray.

The next section comprises an extensive discussion of various types of high-molecular-weight compounds, beginning with the simple inorganic polymers and proceeding to more complex types such as high-polymeric hydrocarbons and rubber, synthetic organic polymers containing esters, amides, ethers, etc.

Carbohydrates are discussed extensively in the light of theories gleaned from study of the relatively simple synthetic polymer systems. Emphasis is given to constitution and structure, to the size, shape, and arrangement of the micelles, to chemical reactions, and to inter-reaction between chains. About 50 pages are devoted to a corresponding discussion concerning starch grains and the starch molecule.

Proteins, including enzymes and viruses, are reviewed in a section of about 150 pages. Here again emphasis is upon the characterization of size, shape, and structure, and upon the effects of these on physical properties. The rubbery state exhibited by certain proteins is described in relation to the earlier discussed elasticity and extensibility of rubber itself. Other properties which are analyzed are solubility in water and salt solutions, association, flocculation, hydration, and denaturation.

Solubility is considered by the author as deserving a section in itself. Here the effects of specific groups, chain length, pH, salts, and crystallinity are described as factors affecting solution. Fractional precipitation and gelation are described. A thermodynamic theory of solutions applied to high polymers is collected from the scattered literature and combined in the terminology of Lewis and Randall.

Films, foils, and membranes, and the molecular structure of animal and plant tissues are discussed in the closing chapters.

The author's approach to the chemistry of proteins and carbohydrates is unique, when compared with that of the usual texts on these subjects. It brings to the biochemist that fundamental physical chemistry which has been developed so successfully in the realm of synthetic polymers, and which is applicable to the more complex biological systems. In such a comprehensive treatise it has been impossible to cover any subject thoroughly; however, an abundance of references has been discriminately chosen for those wishing more details. This book is well written by an author who shows an intelligent and critical mastery of the source material.

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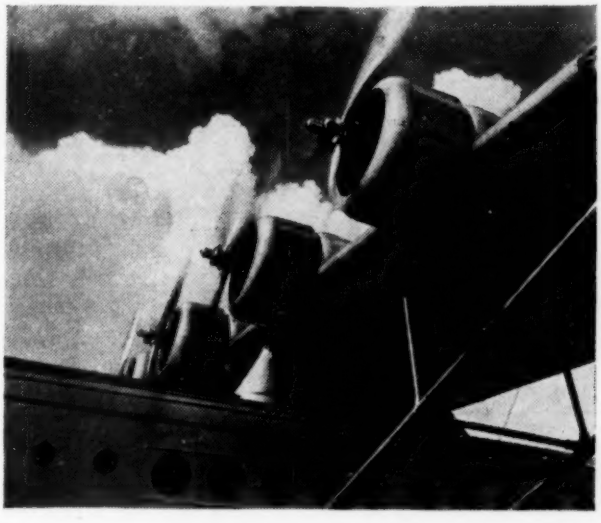

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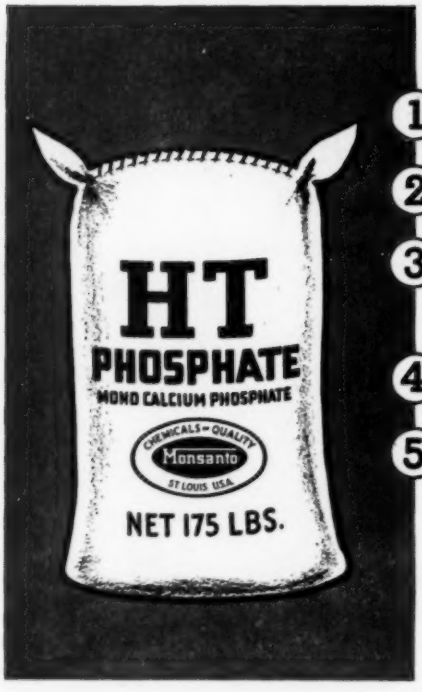

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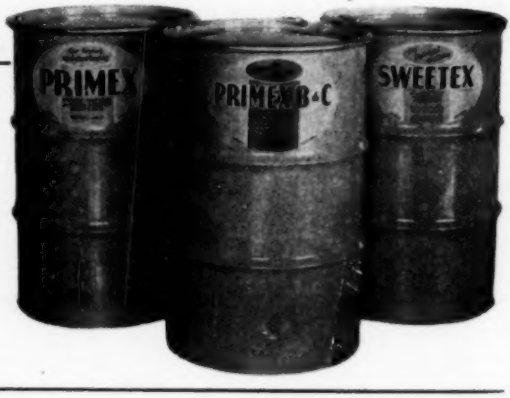

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